QUALITY ASSURANCE PROJECT PLAN (QAPP)

FOR SHIPBOARD TESTING

April. 2012

Prepared by
<<Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University>>

Ballast Water Detecting Lab of Shanghai Ocean University

Compiled by: Wang Qiong 2012.4.1	**Name:**	Qiong Wang
	Title:	Quality Manager
	Date:	
	Singed:	
Approved by: Wu Huixian 2012.4.1	**Name:**	Huixian Wu
	Title:	Technical director
	Date:	
	Singed:	
Reviewed by: Xue Junzeng 2012.4.1	**Name:**	Junzeng Xue
	Title:	Lab supervisor
	Date:	
	Singed:	

Table of Contents

List of Tables

List of Figures

1. Project Management

This QAPP describes the implementation of quality assurance and quality control activities during the evaluation of the PACT marine™ Ballast Water Management System (BWMS) according to the requirements stated in the IMO Guidelines for Approval of Shipboard Ballast Water Treatment Systems: Guidelines for approval of ballast water management systems (G8).

1.1 Title and Approval Sheet

See page 1.

1.2 Table of Contents

See pages 2-5.

1.3 Distribution List

The following is a list of persons who will receive copies of the approved QA Project Plan and any subsequent revisions（table 1）.

Table 1 QAPP Distribution List

QAPP Recipient	Project Role	Organization	Contact Information
Junzeng Xue	Lab supervisor	BWDL of Shanghai Ocean University	Email: jzxue@shou.edu.cn Phone: 86-15692166680
Huixian Wu	Technical director	BWDL of Shanghai Ocean University	Email: hxwu@shou.edu.cn Phone: 86-15692166682
Qiong Wang	Quality manager	BWDL of Shanghai Ocean University	Email: qwang@shou.edu.cn Phone: 86-13122849272
Jiqiang Yang	Sampling personnel/Testing personnel	BWDL of Shanghai Ocean University	Email: 1137100288@qq.com Phone: 86-18301977656
Xiaolin Li	Sampling personnel/Testing personnel	BWDL of Shanghai Ocean University	Email: 260955213@qq.com Phone: 86-15216835202
Liang Liu	Testing personnel/Sampling personnel/Document manager	BWDL of Shanghai Ocean University	Email: ll_laugh@126.com Phone: 86-18817565011
Lin Yuan	Synthesizer/Testing personnel/Instrument manager	BWDL of Shanghai Ocean University	Email: lyuan@shou.edu.cn Phone: 86-13818389140
Yvonne Xia	Technical director	PACT Enviornmental Technology Co., Ltd.	Email: yvonne@pactchina.com Phone: 97226518388
Le Zhang	Document manager	PACT Enviornmental Technology Co., Ltd.	Email: zhangle@pactchina.com
Xueting Lin	Technical manager	PACT Enviornmental Technology Co., Ltd	Email: linxueting@pactchina.com

1.4 Project/Task Organizations

1.4.1 Ballast Water Detecting Lab of Shanghai Ocean University ---- Testing Organization

Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University was founded in September 2008. There are twenty-two persons in the lab, among which four persons are engineers and scientists with high professional title. The lab consists of sample acceptance room, hydrochemistry room, microorganism testing room, microscope room, balance room and sample storage room. The lab is dedicated to the study of the harbor ecology and invasion ecology, mainly of the ecology research study of the plankton in harbor area and ship ballast water and the microorganisms in ocean environment. This organization has published over 200 papers in both national and international academic journals. In addition, the lab has obtained twenty patents authorizations.

The lab is equipped with all kinds of instruments and apparatus, such as BOD_5 analyzer, TOC analyzer, spectrophotometer, stereoscopic microscope, conductivity gauge, turbid meter for water micro-organism test, environmental parameters detection and plankton test. The related staffs are asked to be trained before he or she conducts the testing task. The six doctors and sixteen masters are all specialized in the parameter field. By now, BWDL is able to test five organism indicators and ten water quality parameters in accordance with the ballast water discharging standards regulated in the International Convention for the Control and Management of the Ships' Ballast Water and Sediments: (1) viable organisms greater than or equal to 50 μm in minimum dimension; (2) viable organisms less than 50 μm and greater than or equal to 10 μm in minimum dimension; (3) toxicogenic *Vibrio cholera* (serotypes O1 and O139); (4) *Escherichia coli*; (5) *Intestinal Enterococci*; (6) heterotrophic bacteria; (7) total residual oxidants (TRO); (8) dissolved oxygen (DO); (9) total suspended solids (TSS); (10) turbidity (NTU); (11) dissolved organic carbon (DOC); (12) particulate organic carbon (POC); (13) pH; (14) salinity; (15) temperature. Being realistic and creative, BWDL aims to build a competent and famous lab which is specialized in the testing of ships' ballast water in China.

An organizational chart for the project is shown in Figure 1.

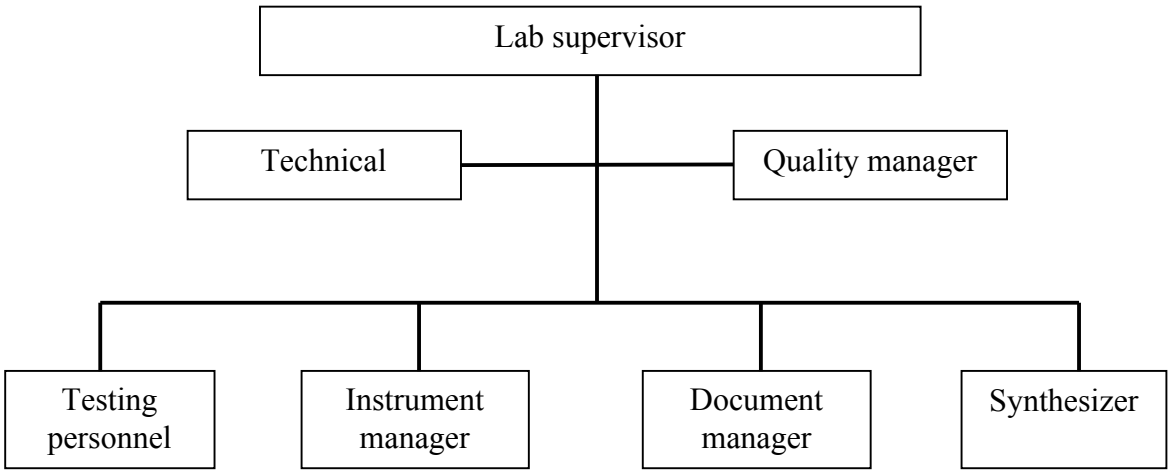


Figure 1. Organizational Chart of the Testing Organization

Lab supervisor: Junzeng Xue

He is responsible for planning and leading the overall project agenda; keeping in touch with PACT Environmental Technology Co., Ltd; approving quality system documents and standard operating procedures (SOPs); and making all final decisions on shipboard testing. He is also BWDL's senior zooplankton scientist and is responsible for the supervision of BWDL technicians in the implementation of relevant SOPs.

Technical director: Huixian Wu

She is BWDL's senior microbial scientist and biological research team leader. She is responsible for the overall technical works of this project; development and implementation of the microbial-related SOPs; training relevant testing personnel for appropriate microbial sample collection and handling, and microbial samples analysis according to relevant SOPs and reviewing all testing results.

Quality manager: Qiong Wang

She is responsible for development and maintenance of BWDL's Quality Management Plan (QMP), this Quality Assurance Project Plan (QAPP) and phytoplankton SOPs. She is also responsible for dealing with the customer complaints, and protection work related to the safety, health and environment. She also plays the role of lab internal affairs supervisor.

Testing personnel/Sampling personnel: Xiaolin Li

She is responsible for filling in sample label; sampling parameters of water qualities; analyzing organisms ≥10 μm to <50 μm; filling in tables of sampling record and original records of the viable organisms ≥10 μm to <50 μm.

Testing personnel/Sampling personnel: Jiqiang Yang

He is responsible for filling in sample label; sampling organisms ≥50 μm; processing the sample pretreatment for TSS and POC analysis; testing salinity and temperature in situ; filling in tables of sampling record, chain of custody form, and original records of water qualities testing.

Testing personnel/Sampling personnel/Document manager: Liang Liu

He is responsible for filling in sample label; sampling organisms ≥10 μm to <50 μm; analyzing bacteria samples in situ; filling in tables of sampling record, original records of bacteria. As document manager, he is responsible for the classification, cataloging and custody of the documents. He is responsible for the filing and managing of test reports and documents related. One of his other responsibilities is to file and manage the technical documents such as standards, regulations, procedures and system documents and the personnel technical documents as well. Moreover, he is asked to keep the file room safe and clean and ensure that the documents in good conditions. He is also the

sample keeper and responsible for the classification and record of the sample. He is responsible for keeping the environment of the sample room in normal condition and he should make the sample room safe and clean and also keep the samples in good condition. He is responsible for the distribution of the test reports in time.

Synthesizer/Testing personnel/Instrument manager: Lin Yuan

He is responsible for filling in sample label; sampling bacteria; analyzing organisms ≥50 μm; filling in tables of sampling record, original records of the viable organisms ≥50 μm. As instrument manager, he is also responsible for the maintenance of the instruments, keeping operation record and maintenance record of the instrument, the calibration of the instrument and preparation and custody of the instrument record card.

1.4.2 PACT Environmental Technology Co., Ltd ---- Vendor

PACT is an equipment manufacturer and contractor in Ballast Water Treatment, established in 1998.
PACT combines decades of experience in design, manufacture and operation of water and wastewater treatment plants in a variety of industries. PACT design and process engineers are a select group of professionals that have gained their experience operating and/or managing major industrial water and wastewater treatment plants.
PACT has been certified with ISO 9001:2008 by China Classification Society (CCS) and establishes, implements, maintains and continually improves Quality Management System according to ISO 9001:2008.
PACT is headed at Shanghai with our development, sales and technical depts.. PACT's facilities locate at Wuxi and Shanghai. Good technical support and after sales service assure our good service to the clients. We provide price competitive product and good after sales service through international cooperation. Currently, we have set service offices in Hong Kong, Southeast Asia, Dubai, Greek, etc. for Ballast Water Treatment.

PACT marine™ BWMS consists of:
Filter – 40um self-cleaning filter
UV System – medium pressure UV(MPUV)
Control System – UV power panel, control panel and online monitoring instrument (including flow meter, light intensityprobe and temperature transmitter)

An organizational chart for the project is shown in Figure 2.

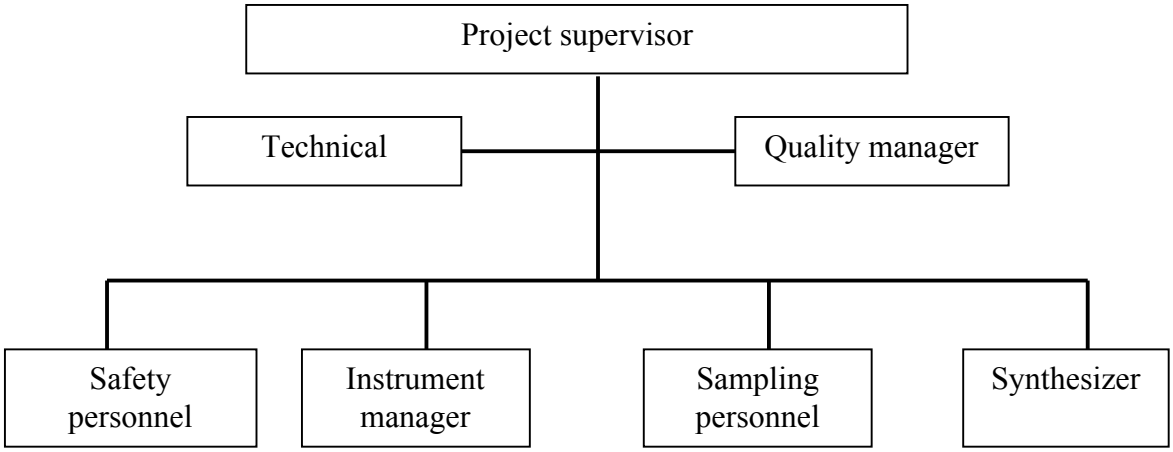


Figure 2. Organizational Chart of the Research and Development Organization

Project supervisor: David Leung

The project supervisor is responsible for the overall management work of the project. It is the responsibility of him to organize the human resource; material resource and financial resource of the company to ensure that the project goes on wheels. He is responsible for establishing the quality policy and quality objectives and arranging the work schedule of the project, and also he has the responsibility for urging the staff of the project to follow the requirements of management system files and regulations of the company.

Technical director: Yvonne Xia

The director is responsible for the overall technical works of the project, and he is responsible for the technical training of the personnel involved in the related test work, arranging the experimental flow and technical principle studying for the related staff. Organizing and coordinating the development of the test is also one of his responsibilities. He is also in charge of dealing with the emergencies occurred during the test process. Moreover, he is responsible for the assurance of test tempo and device status to be in compliance with the requirements of the QAPP.

Quality manager: Jianhua Gu

He is responsible for the quality related work and ensures that the quality objectives to be fulfilled. In addition, he is in charge of the safety, health and environmental protection work throughout the development of the project. He is responsible for supervising the staff of the project team to finish the work in accordance with the QAPP.

Safety personnel: Kevin Wang

He is responsible for the safety of the test field. Keep eye on the safety of the test field, give suggestions on how to deal with the potential safety hazard and monitor the implementation of the improvement measures and ensure the test running in order.

Instrument manager: Le Zhang

The operation of the ballast water management system in conformation with the requirements in the test process is one of his responsibilities. And keep record of it. Report to the technical director about the running status of instruments and help the technical director with solving the defaults of the instruments.

Synthesizer: George Qiu

He is responsible for the management of the document and files over the whole course of the project. Organize and store the documents according to the requirements. He is also in charge of the coordination of the resources in field test.

Sampling personnel: Yihua Ren

He is responsible for assisting the test organizations in collecting the samples. Ensure that the samples are classified and managed in order.

1.5 Project Background

With the rapid development of the world trade and global tourism, the demand for freedom trade is growing and the marine shipping industry is exuberant and occupies 60% share of world trade. To assure the safety of sailing ships, it is necessary to add some ballast to keep the ship in an appropriate stable and floating status. Since 1980s, it is common to use water as ballast, and it is the so called ballast water. While the ballast water makes it easier for the spread of species from one water region to another one. Once ballast water in ship containing harmful aquatic organisms or pathogens is discharged to the waters of another port state, it will endanger the local ecology, economy and human health, and the effect will last for a long time. Once the aquatic organisms invade and inhibit in the local waters, they will reproduce in an uncontrollable manner, then destroy the food web of local species. And these disastrous causes will lead to mass propagation of harmful parasite and pathogen and even extinguish the local species.

The test and management of ballast water is getting more and more important as the ocean pollution is getting worse and worse due to the ships' ballast water discharge. Aiming to prevent the potentially devastating effects of the spread of harmful aquatic organisms and pathogens carried by ships' ballast water from one region to another. IMO proposed and approves the International Convention for the Control and Management of the Ship's Ballast Water and Sediments (hereinafter BWM Convention) in 2004. The BWM Convention requires that: (1) the average density of organisms greater than or equal to 50 micrometers in minimum diameter in the replicate samples is less than 10 viable organisms per cubic meter; (2) the average density of organisms less than 50 micrometers and greater than or equal to 10 micrometers in minimum diameter in the replicate samples is less than 10 viable organisms per milliliter; (3) the average density of *Vibrio cholerae* (serotypes O1 and O139) is less than 1 cfu per 100 milliliters, or less than 1 cfu per 1 gramme (wet weight) zooplankton samples; (4) the average density of *E. coli* in the replicate samples is less than 250 cfu per 100 milliliters; (5) the average density of intestinal *enterococci* in the replicate samples is less than 100

cfu per 100 milliliters.

According to Guidelines G8 of the IMO, the approval of ballast water management systems are required to perform series of testing on board, in order to assess whether ballast water management systems meet the standard as set out in regulation D-2 of the BWM Convention.

1.6 Description of Vessel and Technology Set-up

1.6.1 Vessel

The information of the test vessel is showed in the table below.

Table 2 Particular of the Test Vessel

Vessel particular	
Name	Hongtai 158
Owner	Rizhao Eastern Horizon Shipping Co., Ltd
Capacity of the ballast pump	450 m^3/h
Ballast tanks	4500m^3

1.6.2 PACT marine™ Ballast Water Management System (BWMS)

1.6.2.1 System Components and Their Functions

PACT marine™ P-300 is consisted mainly by:

（1）Filter --- a 40μm self cleaning filter. The filter size: 2769mm(L) *610mm(¢)
（2）UV System --- a medium pressure UV（MPUV）.
UV size: 925(L) mm *680 (W) mm* 651(H)mm
（3）Control System --- an UV switch board, a control panel and online monitoring instrument（including flow meter, light intensity probe and temperature transmitter）. HMi soft uses wincc flexible 2008。MCC size 1000(L) mm * 600(W) mm * 1900(H)mm； PLC size 600(L) * mm* 350(W) mm *1500(H)mm

Nominal Voltage/Power of the whole system assembled: 220v，50kw；380v，5kw

Considering the system assembling, especial flexibility of resembling, the filter and UV system in PACT marine™ are separated. The footprint of the filter is larger and the UV system can be assembled anywhere after the filter and before deballast treatment.

The UV system, as essential, can be installed the existed pipes without any holder. The system can be assembled horizontally and vertically and its performance won't be effected accordingly.

The general arrangement drawing of the system is as shown in Figure 3.

Figure 3. PACT marine™ BWMS

1.6.2.2 Working Procedure

Generally, when ballast water is loaded in the ship, the seawater will flow into the sea chest, then pass a mechanical filter to remove bigger organisms and substances, and flow into the Ballast Water Treatment (BWT) last.

Ballast Process：

When ballasting, seawater will pass a coarse filter to remove bigger organisms and substance primarily, then pass a filter F-01 in Ballast Water Treatment (BWT) to remove organism larger than 40μm and solid particles. The filter can be self cleaned according to PLC without breaking off filtering. The solid particles and organisms filtered by the filter would flow into backflushed water when the filter is backflushed and then be discharged into local ocean. Therefore, active ocean organisms and sedimentation in the ballast tank are reduced and then the living and growing probability of ocean organisms in the ballast tank are reduced accordingly. The ballast water after being filtered will pass the UV System UV-01 and then flow into the ballast tank.

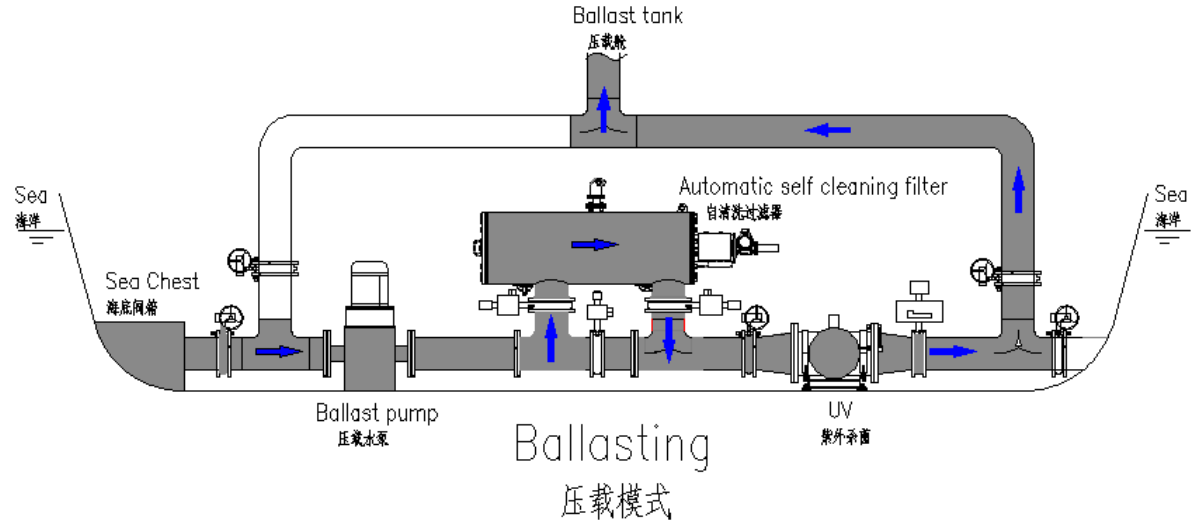


Figure 4. PACT marine™ BWMS Ballast Process

Discharge Process：

When discharging, the ballast water in the ballast tank will not pass the filter but the UV System and then be discharged after treatment by the UV System. Considering the probability of ocean organisms' living and reliving by light, the ballast water should be sterilized again before discharging. PACT marine™ can realize statistics auto collection and auto recording, including flow rate, light intensity and temperature.

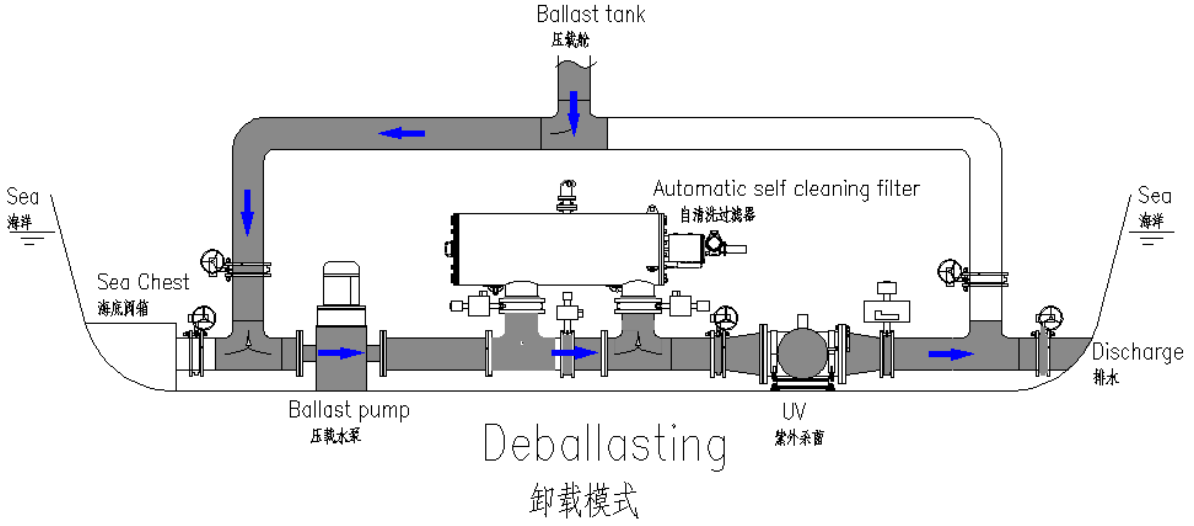


Figure 5. PACT marine™ BWMS Deballast Process

1.7 Special Training

1.7.1 Training for Sample Collection and Handling

Training is provided to those individuals that are required to collect and/or handle samples in accordance with the Guidelines for Ballast Water Sampling G2 and Specification for Marine Monitoring (GB17378-2007). BWDL senior scientist and experienced staff are responsible for ensuring that technicians under their supervision

are adequately trained in applicable procedures and requirements.

1.7.2 Training for Analysis

The test and analysis staff of the lab should be trained according to the Personnel Training Procedures of BWDL of Shanghai Ocean University; Specification for Marine Monitoring (GB17378.4-2007 and GB17378.7-2007), Gulf Ecosystem Observation Method (2005); Standard Examination Methods for Drinking Water-Indicator Microbes (GB/T 5750.12-2006); Diagnostic Criteria for *Vibrio Cholera* (WS 289-2008); Water quality-Detection and Enumeration of intestinal *enterococci* - Part 2: Membrane Filtration Method (ISO 7899-2:2000); Guidelines for Approval of Ballast Water Management Systems G8； General requirements for the competence of testing and calibration laboratories (ISO 17025-2005).

Testing personnel who will undertake the chemical related work in the lab should learn how to protect and rescue themselves. The important chemical test staff who know well of the test methods, procedures, objectives and result assessment should master the assessment method for determination of uncertainty of environmental parameters analysis.

The organism test staff should know the safe handling and sterilization procedures of organism test. All the staff should be assured to be qualified and supervised to carry out the work according to the management system. The lab manager should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment, test report sign and certificate verification.

1.8 Documentation and Records

1.8.1 QAPP

BWDL and PACT will discuss and determine the QAPP prior to the implementation of the project. And the approved QAPP will be handled and recorded as pre the project controlled document.

1.8.2 Field Running Record

To make sure that the system operates within the normal parameter range in the test cycle, running status of the ballast water management system is recorded (Table 3)

Table 3 Running Record of PACT marine™ BWMS

Entrustment organization				
Test equipment				
Test location				
Test cycles			Test date	
Test phase	ballasting □ holding □ deballasting □			
Start time			End time	
Supervision unit			Supervisor	
Test related information	Equipment running status			
	Running parameter	Flow: Dose:		
	Sampling related information	Biological efficacy water sample □ Relevant chemical water sample □		
Recorder:		Quality manager:		Project supervisor:

1.8.3 Field Sampling Record

Information about the collected sampling data will be recorded on the Sample Label and the information in tables is about sample lot number, the date, environmental condition, sampling point, sampling personnel, and supervisor. Any deviations from the standard sample procedure or emergencies should be recorded. The sample label is shown below (Figure 6).

Sample ID:	
Sampling Time:	Sampling temperature:
Sampling humidity:	Sampling point:
Sampling personnel:	Supervisor:

Figure 6. Sample Label

The sampling record table is shown in Table 4.

Table 4 Sampling Record

Sample name			
Vessel name		Client	
Sampling personnel		Sampling date	
Number of samples		Environmental factors	Temp.: °C Humidity: %RH
Port registry		Distinctive number or letters	
Ship's tonnage		Ballast water volumes	
Shipping date		Sample ID	
Ballast tank type and location		Ballast water treatment technology	
BWMS composition		Sampling method	
Ballast tank volume		Other sampling method	
Sample type	organisms ≥50 μm □ organisms 10~50 μm □ organisms <10 μm □	Net (Vertical height of trawl, diameter, mash)	
Pump (depth, flow rate)		Bottle (sampling depth, volume)	
Beginning of sampling time		End of sampling time	
Sampling volume		Sampling location	Intake □ Discharge □
Sampling port	Intake □ Discharge □	Size of net (μm)	
Sampling results	Recorder:		Date:

1.8.4 Chain of Custody Form

Chain-of-custody procedures are strictly followed for all samples so that the possession of a sample is traceable and documentable from the time of its collection until the time of its analysis.

For samples under Chain-of-Custody, a Chain-of-Custody form (Table 5) must

accompany each shipment (i.e., one form can accommodate multiple samples).

Table 5 Chain of Custody Form

Test task number		Custody date	
Test item		Custody personnel	
Test objective			
Test personnel			
Custody contents			
Contract (letter of authorization)	Complete □ Lack of contents □ No □		
Test plan	Complete □ To be □ No □		
Instrument	Calibration status qualify □ Allow to use □ Forbidden to use □		
Personnel	Qualification Hold qualification card □ No qualification card □		
Sample	Quantity		
	Appearance quality Qualified □ Unqualified □		
	Unique mark Have □ No □		
	Sample preparation record Have □ No □		
Test method	Right □ To be completed □ Problem □		
	Standard (regulation) Have □ No □		
Environment	Temperature °C	Humidity %RH	
	Condition: Qualified □ Unqualified □		
Record	Original record: Meet the requirement □ Do not meet the requirement □ No record □		
	Operation record: Meet the requirement □ Do not meet the requirement □ No record □		
Response action of custody			
Remarks			

1.8.5 Laboratory Original Data Records

The original data in the lab must be recorded clearly, and the records will be stored in an appropriate facility to keep them away from damage or losing, and also be accessed easily. Preservation period of the record must be specified and all the records should be kept secret. The collection, retrieval, access, file, storage, maintenance and cleaning of the quality record and technical record should be in compliance with the Control Procedures of Ballast Water Detecting Lab of Shanghai Ocean University.

The lab should preserve the detailed records of the information about original observation, educe of data, verification route, calibration records, personnel record and the copies of report distributed within the stipulated preservation period. Information

such as sample number, test date, standards, test conditions and so on should be included on each test record or calibration record: This would be of great help to identify the factors of uncertainties and assure the repeatability of the test or calibration in conditions similar to the original. The content of the records should involve information about the name of the sampling personnel, the test personnel, verification personnel. If culture medium is prepared, it is necessary to make a record of the name and type of the culture medium; marks of the preparation time and preparation personnel; culture medium/solution type and volume; volume of the sub-package; composition, content, manufacturer and lot number of each composition; pH value (initial and final); complementing means, time and temperature of sterilizing measures etc. should be recorded. Keep record of the observation result, data and calculations in situ, and identify the records according to the requirements of specified tasks.

If the lab records need to be modified, two lines should be written on the original records, and don't erase the original records. Then the modified records should be written near the original records with the mender's stamper or signature or abbreviated signature.

Tables of original record of the phytoplankton, zooplankton, bacteria test and water qualities are shown in Appendices A-H, respectively.

2. Experimental Approach

2.1 Navigation Route

We report here on system performance tests during a transition cruise from Zhangjiagang port,China, to Tianjin Port, China, acrossing Donghai sea, Huanghai sea, Bohai sea, between September 2013 to April 2014.

Samples were taken from Donghai sea near Huanghai sea, different zones of Huanghai sea, Bohai sea. The shipboard tests (five in total) were performed per below table.

Table 6 The information of ship route

Test cycle	Opration	Sampling location	Sampling date
013	Ballast	East China Sea	2013.09.15
	Deballast	North Yellow Sea	2013.09.17
014	Ballast	South Yellow Sea	2013.10.11
	Deballast	Bohai Sea	2013.10.13
015	Ballast	East China Sea	2013.10.27
	Deballast	Middle Yellow Sea	2013.10.29
016	Ballast	Middle Yellow Sea	2013.10.29
	Deballast	Bohai Sea	2013.10.31
017	Ballast	South Yellow Sea	2014.04.03
	Deballast	Bohai Sea	2014.04.05

The PACT marine™ BWMS was tested extensively across multiple geographic areas and a variety of water conditions.

2.2 Test Set-up

The test set up is mainly composed of the ballast pump, the PACT marine™ BWMS, the treated tank, the control tank and the sampling facility. The test equipment (PACT marine™ BWMS) is connected to the ship's piping system by valves and pipes (Figure 7).

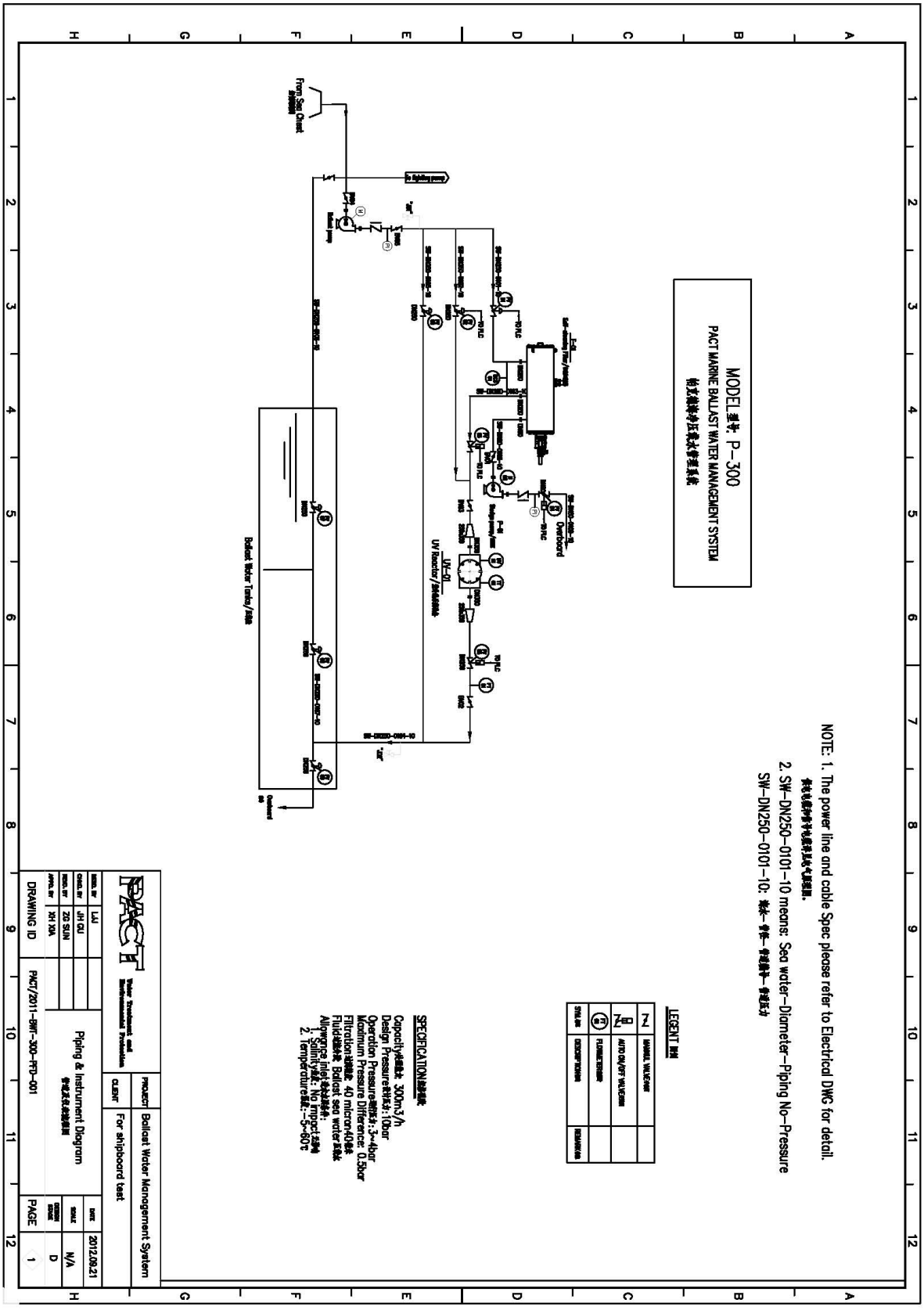


Figure 7. Diagrammatical Drawing of the Test Set-up

The test set-up is representative of the real ballasting and deballasting process. The ballast water is pumped by the ballast pump and flows through the ballast water management system and then enters the ballast tank. The untreated ballast water will enter the control tank by the bypass pipe. When discharging, the ballast water in both the treated tank and the control tank is discharged to the overboard directly.

Information on the model of the tested equipment is shown below:

Model	Ballast pump	Treated tank	Control tank
P-300	450 m^3/h	419m^3	620m^3

To ensure that the PACT marine™ BWMS is of a capacity within the range of the treatment rated capacity for which it is intended, the capacity of the ballast pump in the test set-up is 450 m^3/h which is higher than the treatment capacity of the tested equipment. When the test is conducted, the flow of seawater is splited at the outlet of the ballast pump, with one flow to the test set-up by pipe, and the other be discharged over board by the branch pipe.

During test, the capacity of the ballast water to be uptaken or discharged would with reference to the navigation route, the weather and the sea conditions.

The sampling facility named S is installed on the ballast pipe to collect the uptake water sample, the control water sample, the discharge sample from the treated tank and the discharge sample from the control tank. The design of the sampling point is in accordance with requirement in G2; the structure of the sampling facility is shown in Figure 8.

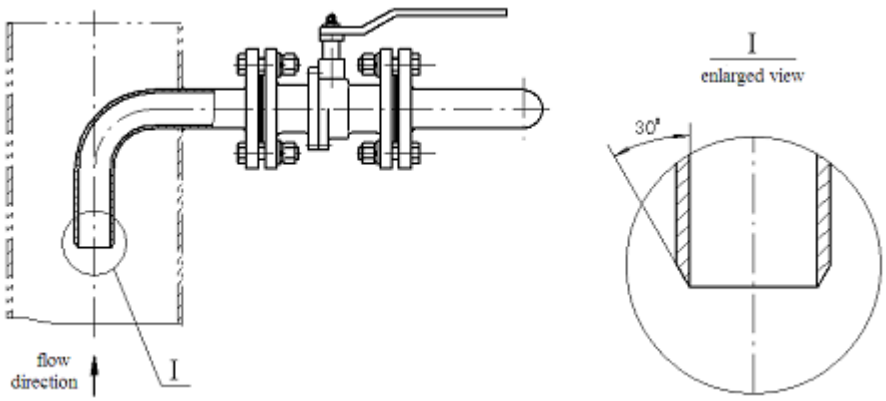


Figure 8. Sampling Facility

2.3 Technology Installation and Commissioning

Verify that the installation of the ballast water management is in compliance with the installation specifications. The installation process is in accordance with the rules of the Society. The operational inlets and outlets are located in the positions indicated on the

drawing of the P&ID.

Commissioning test will be conducted after the ballast water management system is fitted on the ship, the commissioning test includes test to the control and monitor equipment, the safety alarming test and the running test.

2.4 Performance Criteria for Influent Water and Discharge Water

2.4.1 Influent water

Influent water used for testing is representative of harbor or coastal waters. Testing must include temperate, semi-tropical, or tropical locations with ambient organism concentrations that will provide a significant challenge to the efficacy of the BWMS: (1) influent water ≥50 μm size exceeds 100 organisms per cubic meter; (2) influent water 10~50 μm size exceeds 100 organisms per cubic millimeter.

The source water for test cycles will be characterized by measurement of salinity, temperature, particulate organic carbon (POC) and total suspended solids (TSS).

2.4.2 Discharge water

The biological efficacy testing of the BWMS will be assessed during the shipboard test according to the Guideline G8. Control tank viable organism concentration exceeding the values of regulation D-2.1 on discharge. The concentration of organisms and bacteria in the discharge treated water should meet the standard as set out in regulation D-2: (1) less than 10 viable organisms per cubic meter greater than or equal to 50 micrometers in minimum dimension; (2) less than 10 viable organisms per milliliter less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension; and (3) less than the following concentrations of indicator microbes, as a human health standard: (a) toxicogenic *Vibrio cholerae* (serotypes O1 and O139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gramme (wet weight) of zooplankton samples; (b) *Escherichia coli* less than 250 cfu per 100 milliliters; and (c) Intestinal *Enterococci* less than 100 cfu per 100 milliliters.

2.5 Running Test Cycles

The test cycles including invalid and unsuccessful test cycles conducted to the test equipment which is the model PACT marine™ P-300 BWMS are to span a period of not less than three months. At least three consecutive test cycles that comply with regulation D-2 will be performed. Any invalid test cycle does not affect the consecutive sequence. A complete shipboard test cycle includes uptake of ballast water of the ship, storage of ballast water on the ship, treatment of the ballast water by the BWMS, and discharge of ballast water from the ship.

The amount of ballast water tested in the test cycle on board should be consistent with the normal ballast operations of the ship and the BWMS should be operated at the treatment rated capacity for which it is intended to be approved. Documentation that the BWMS is of a capacity within the range of the rated treatment capacity for which it is

intended.

2.6 Sampling and Measurements

2.6.1 Sampling Points Arrangement

In a whole test cycle, the influent water samples are taken during ballast water uptake. Sampling ports are located as close as practicable to the BWMS prior to treatment to determine concentrations of living organisms upon uptake.

The discharge samples from both the treated tank and the control tank are taken when discharging. Sampling ports are located as close as practicable to the BWMS overboard outlet prior to the discharge point to determine concentrations of living organisms prior to discharge.

The sampling is performed by qualify personnel. The arrangement of the sampling point is shown in Figure 9. S1 will be three replicate samples of influent water, collected over the period of uptake (e.g., beginning, middle, end); S2 will be three replicate samples of influent control water, collected over the period of uptake (e.g., beginning, middle, end)S3 will be three replicate samples of discharge treated water collected at each of three times during the period of discharge (e.g., 3 × beginning, 3 × middle, 3 × end); and S4 will be three replicate samples of discharge control water, collected over the period of discharge (e.g., beginning, middle, end).

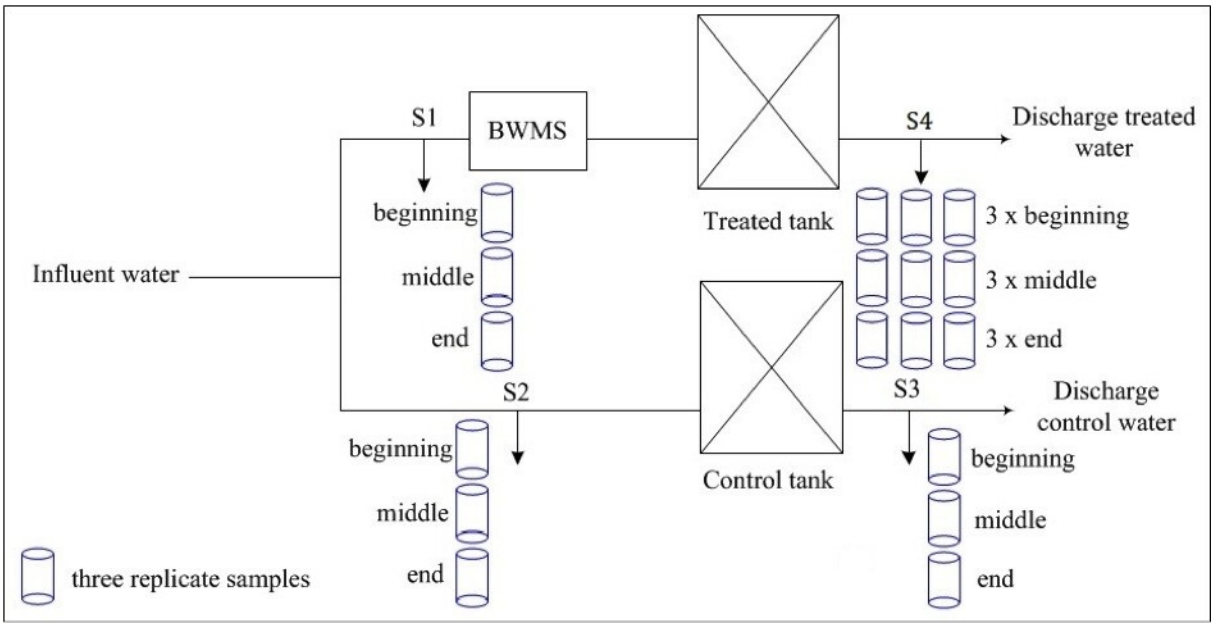


Figure 9. Arrangement of the Sampling Points

2.6.2 Sampling Process Design

Samples are collected either continuously during or at the beginning, middle, and end of the period of ballast water uptake and discharge. In cases when the treatment capacity is 300 m^3/h, the total time needed for one test cycle is 60 min. Table 7 shows the

detailed time for sampling arrangement.

Table 7 Sampling Phase Arrangement

Sampling point	Sampling time			Remarks
S1	10 min	30 min	50 min	The previous 10 min is taken to wash the pipes.
S2	10 min	30 min	50 min	The previous 10 min is taken to wash the pipes.
S3	10 min	30 min	50 min	The previous 10 min is taken to wash the pipes.

Table 8 summarizes all types of measurements to be taken during the study. Grab samples for biological test and water quality test will be collected in triplicates. Procedures for sampling and transfer are described in detail in section 3.

Table 8 Parameters to be measured during the study; time and type of sampling and measurement location

Parameter	Sample ports	Type of sample	Location
Organisms ≥50 μm	S1, S2, S3, S4	In situ, Discrete grab	Hongtai 158; BWDL, Shanghai
Organisms ≥10 ~ 50 μm	S1, S2, S3, S4	In situ, Discrete grab	Hongtai 158; BWDL, Shanghai
Organisms <10 μm	S1, S2, S3, S4	In situ, Discrete grab	Hongtai 158
Salinity	S1, S2, S3, S4	In situ, Discrete grab	Hongtai 158
Temperature	S1, S2, S3, S4	In situ, Discrete grab	Hongtai 158
Total suspended solids (TSS)	S1, S2, S3, S4	Discrete grab	Hongtai 158; BWDL, Shanghai
Particulate organic carbon (POC)	S1, S2, S3, S4	Discrete grab	Hongtai 158; BWDL, Shanghai

2.7 Time Schedule for the Testing Period

Table 9 Time schedule

Task name	Week No.																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
QAPP preparation																							
Test preparation																							
BWMS installation																							
Test cycle 1																							
Test cycle 2																							
Test cycle 3																							
Test cycle 4																							
Data acquisition																							
Data validation																							
Test reports																							

3. Sampling Protocols

3.1 Sample Collection

First microbiological samples are collected and then samples for water quality. Table 10 summarizes which sampling equipment is used to collect samples for the individual parameters.

Table 10 Equipment and containers used for sampling and sampled volume for the individual parameters

Parameter	Sampling equipment	Sample container	Collected volume S1, S2, S4	Collected volume S3
Organisms ≥50 μm	Directly	Clean HDPE bottle	3 m^3 (3×1 m^3)	9 m^3 (3×3×1 m^3)
Organisms ≥10 ~ 50 μm	Directly	Clean HDPE bottle	30 L (3×10 L)	90 L (3×3×10 L)
E. coli	Directly	Clean glass bottle	300 mL (3×100 mL)	900 mL (3×3×100 mL)
Intestinal *Enterococci*	Directly	Clean glass bottle	300 mL (3×100 mL)	900 mL (3×3×100 mL)
Heterotrophic bacteria and *Vibrio cholerae*	Directly	Clean glass bottle	1500 mL (3×500 mL)	4500 mL (3×3×500 mL)
Salinity	Directly	Clean HDPE bottle	3 L (3×1 L)	3 L (3×1 L)
Temperature	Directly	Clean HDPE bottle	3 L (3×1 L)	3 L (3×1 L)
TSS	Directly	Clean HDPE bottle	3 L (3×1 L)	3 L (3×1 L)
POC	Directly	Clean glass bottle	3 L (3×1 L)	3 L (3×1 L)

The procedures for collecting samples from the different sampling ports and sampling times are as follows:

1) Sampling of organisms ≥50 μm from sampling ports S1, S2, S4. Organisms with a minimum diameter larger than 50 μm are sampled as 3 × 1 m^3. The water is directly sampled from sampling ports and the water is slowly sieved through a plankton net (50 μm diagonal dimensions). The remained organisms on the net are washed by filtered water for at least three times. Collect the organisms in a 100 mL bottle. The sieved water is controlled by a flow meter to ensure accurate sampling volume. Consider of the higher density, the concentrated sample volume should be adjusted.

2) Sampling of organisms ≥50 μm from sampling ports S3. Organisms with a minimum diameter larger than 50 μm are sampled as 3 × 3 × 1 m^3. The water is directly sampled from sampling ports and the water is slowly sieved through a plankton net (50 μm diagonal dimensions). The remained organisms on the net are washed by filtered water

for at least three times. Collect the organisms in a 30 mL bottle. The sieved water is controlled by a flow meter to ensure accurate sampling volume.

3) Sampling of organisms 10~50 μm from sampling ports S1, S2, S4. Organisms with a minimum diameter between 10 and 50 μm are sampled as 3 × 10 L with a 10-L water sampler. The sampled water is slowly sieved through a plankton net (10 μm diagonal dimensions). The remained organisms on the net are washed by filtered water for at least three times. Collect the organisms in a 100 mL bottle.

4) Sampling of organisms 10~50 μm from sampling ports S3. Organisms with a minimum diameter between 10 and 50 μm are sampled as 3 × 3 × 10 L with a 10-L water sampler. The sampled water is slowly sieved through a plankton net (10 μm diagonal dimensions). The remained organisms on the net are washed by filtered water for at least three times. Collect the organisms in a 100 mL bottle.

5) Sampling of bacteria from sampling ports S1, S2, S4. *E. coli* samples are collected as 3 × 100 mL grab samples by slowly submerging 100 mL sterile bottles. Intestinal *Enterococci* samples are collected as 3 × 100 mL grab samples by slowly submerging 100 mL sterile bottles. Heterotrophic bacteria *and Vibrio cholerae* samples are collected as 3 × 500 mL grab samples by slowly submerging 500 mL sterile bottles . The bottle is closed immediately after sampling and cooled at 4°C.

6) Sampling of bacteria from sampling ports S3. *E. coli* samples are collected as 3 × 3 × 100 mL grab samples by slowly submerging 100 mL sterile bottles. Intestinal *Enterococci* samples are collected as 3 × 3 × 100 mL grab samples by slowly submerging 100 mL sterile bottles. Heterotrophic bacteria and *Vibrio cholerae* are collected as 3 × 3 × 500 mL grab samples by slowly submerging 500 mL sterile bottles. The bottle is closed immediately after sampling and cooled at 4°C.

7) Sampling of test water for salinity, temperature, TSS and POC from sampling ports S1, S2, S3, S4. Samples are collected as 3 × 1 L grab samples by slowly submerging a 1 L clean plastic bottle. The bottle is closed immediately after sampling.

3.2 Sample Preservation

The bacteria samples are processed immediately after sampling on board and cooled at 4°C until analyze.
After samples of organisms ≥10 μm to <50 μm are collected, add algae staining solution with concentration of 2ml/L to the sediment drum for 15 minutes. Then add formalin to fix the sample. After the samples are taken back to the lab, make the samples static for 24h and later use a device to absorb and filter the supernatant fluid of the phytoplankton. After 80% of supernatant fluid is filtered, make the sample stay static once again. After the sample is secondary absorbed to about 100ml, make it stay static for 24h for the third time, and then a constant volume 50ml is left for microscopic counting.
After sample of organisms ≥50 μm, add five drops of formalin to fix the sample. Then the samples are taken back to the lab, settling down for 24h. After that filter part of the

supernatant fluid and remove the samples to the 50ml cone centrifuge tube and keep making it settle down. Finally, filter the supernatant fluid and microscopic count the total number of the organisms.
After analysis, samples for organisms ≥50 μm and ≥10 μm to <50 μm are fixed with formalin in situ, placed in the dark and delivered to the laboratory.
The bacteria samples are processed immediately after sampling on board and cooled at 4°C until analyze.
Parameters like salinity and temperature are measured in situ immediately after sampling. TSS and POC should be processed within 24 h after sampling and stored in dark. Samples are packed in a cooler bag (4°C) under transport. When the samples arrived at the laboratory, they are stored in a cool room. If POC testing cannot be finished within 24 h, add a small amount of $HgCl_2$ in sample and stored under -20°C for 7 days.

Preservation methods and expected storage/holding times before measurement are shown in Table 11.

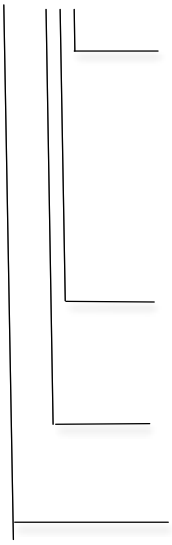
Table 11 Preservation methods and expected storage/holding times before measurement

Parameter	Processing/Preservation	Max. holding time	Expected storage time
Organisms ≥50 μm	Stain with janus green B, and add formalin.	6 month	<6 month
Organisms ≥10 μm to <50 μm	Stain with algae staining solution, and add formalin for preserving sample.	6 months	<6 months
Heterotrophic bacteria *E. coli*, Intestinal *Enterococci*, *Vibrio cholerae*	Process in-situ and store at 4°C until analyze. Enumerate using appropriate media.	6 hours	<6 hours
Salinity	Analyze immediately using salimeter in-situ.	-	0
Temperature	Analyze immediately using thermometer in-situ.	-	0
TSS	Filter immediately after sampling in-situ. Store filter membrane at 4°C.	2 months	<2 months
POC	Store in the dark until processing. Add $HgCl_2$ and store under -20°C.	7 days	0-5 days

3.3 Sample Labeling

The numbering rule of the sample is as below:

SHOU-BWDL-××-×××



Sample number: the samples collected at S1 are marked 1, 2, and 3, represent the beginning, middle and end; the samples collected at S2 are marked 4, 5, and 6, represent the beginning, middle and end; the samples collected at S3 are marked 7, 8, 9, 10,11,12 and 13,14,15 represent the beginning, middle and end; the samples collected at S4 are marked 16, 17 and 18 represent the beginning, middle and end .

For enumeration and/or determination of: A =≥10~50 μm, B =≥50 μm, C = bacteria, D = water quality.

Test cycles: 001 = first test cycle,002 = second test cycle, 003 = third test cycle (if more than three test cycles, the same rule domains, fourth test cycle, fifth test cycle and so on).

The abbreviation of the BWMS manufacturer.

3.4 Sampling/Test System Failure Response and Remedy

Any sampling and testing interruptions or unexpected things occur during sampling should be given a due consideration and causes of them should be found. Report to the technical director in time and keep detailed record of the failure event. If it is resulting from instrument system failure, continue to test by applying another same instrument if necessary. Or else, repair immediately after report to the technical director.

Once the test is interrupted, cut the power supply in accordance with operation procedures.

There are circumstances when external accidents such as power failure, water failure and so on, happen which will affect the testing quality. Therefore, re-sampling and retest are needed when it returns to normal. When it happens that the failure of instruments or apparatus causes the test to breakup. If there is a backup instrument, use it to replace the faulty one and continue the test. If there is only one instrument, and the failure of the instrument will affect the test quality, re-sampling until the instrument returns to normal. The instrument will be handled according to the Procedures for Instrument and Apparatus Management. If something is wrong with the sample, stop the test and report to the technical director. Check the sample, find out the causes and make a suggestion. After being approved, decide how to solve the problem. Re-sampling and re-test are necessary.

Keep record of all abnormities and interruptions occur in the handling process and fill in the sampling/test process and the result abnormalities handling record table. And report to the technical director.

4. Testing and Measurement Protocols

According to Guidelines G8, the lab will conduct the organisms testing including heterotrophic bacteria, *Escherichia coli*, intestinal *enterococci*, Toxicogenic *Vibrio cholerae* (serotypes O1 and O139), organisms ≥50 μm and ≥10 μm to <50 μm, and four environmental parameters, including salinity, temperature, POC and TSS.

4.1 Organisms ≥50 μm

The samples being filtered and concentrated are identified and analyzed by total count method and counted by kind/species to calculate the organism number (number of organisms in per unit)(GB17378.7-2007):

$$r_B = \frac{N_B}{V}$$

,

Where:
r_B——density of organisms ≥50 μm in per unit of volume
N_B——number of zooplanktons (ind);
V——volume of filtered water, unit:(m^3).

Detailed information on organisms ≥50 μm determination is provided in Appendix A.

4.2 Organisms ≥10 μm to <50 μm

Lightly absorb the supernatant fluid from the pretreated samples using a suction pipe with 10μm bolting silk. After settling down for a few times, the water sample is condensed to a 50ml thimble tube. Shake enough before sampling counting, absorb a certain amount of sample and then release it at the counting chamber covered with cover glass (make sure there are no bubbles remain) and then conduct the microscopic counting(GB17378.7-2007).
Optical microscopic counting (concentrated counting):

$$C = \frac{n \times V_1}{V_2 \times V_n}$$

Where:
C——total amount of samples in per unit volume, unit:(cells/mL)
n——number of samples, unit:(cells);
V_1——the volume of concentrated water sample, unit: ml;
V_2——volume of filtered water, unit:(mL);
V_n——volume of sampling counting, unit:(ml)

Detailed information on organisms ≥10 μm to <50 μm determination is provided in Appendix B.

4.3 Organisms <10 μm

4.3.1 Determination of heterotrophic bacteria

Add 1 mL of tween-80 solution to per 100 mL of bacteria sample solution. Gradient dilution is made by high pressure sterilized seawater. Before the water sample is diluted, shake it with effort to make it mixed sufficiently. 10 mL water sample is sucked by a sterilized suction tube and added to 90 mL sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times degree dilution, make the 100 times, 1000 times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. Take 0.1 mL diluted water sample and spread it uniformly on the 2216E culture medium. Four dilution degrees of each water sample are needed to be prepared and replicate two plates for each dilution degree. Put the plate into a constant temperature culture box (25^{o}C) with its upside down for 7 days. Count the total number of the colonies with a stereomicroscope: (1) Do not count when large lawn appears on the plate; (2) Plate with number of colonies between 30 and 300 is selected; the average number of colonies multiplies the dilution degree (10 times, 100 times or 1000 times) equals to the number of the bacteria in water sample; (3) If there are two kinds of dilution degrees with average number of colonies are between 30 and 300, the ratio of the two numbers determines which one to choose. If the ratio is less than 2, the average of the two is chosen; if more than 2, the colony with less number is chosen; (4) If all the average values of all kinds of degrees of dilution are more than 300, the number of colonies is counted using the average number of colony in the largest degree of dilution (lowest concentration) multiplies the times of dilution; (5) If all the average values of all different degrees of dilutions are all less than 30, the number of colonies is counted using the smallest degree of dilution (highest concentration) multiplies the times of dilution; (6) If there are no colonies in all different degrees of dilution, and no inhibitor is tested, then report less than 1 multiplies the lowest diluted times.

Detailed information on heterotrophic bacteria determination is provided in Appendix C.

4.3.2 Determination of *Escherichia coli*

E. coli are quantified according to the specification for marine monitoring-Part 7: Ecological survey for offshore pollution and biological monitoring (GB 17378.7-2007/10.1) and standard examination methods for drinking water-Microbiological parameters (GB/T 5750.12-2006/2.2, 4.2).

With diameter of 0.45 microns of microporous membrane filter to filter water, place the membrane on the lactose and cultivate at 37^{o}C for 24 h, the selective medium were used to form the characteristic colony which is aerobic and facultative anaerobic gram-negative no-sporeforming bacillus.

The membrane of positive total coliforms was cultivated in medium containing fluorescent substrate, which can produce β-glucuronidase that release fluorescent

products because of decomposition of fluorescent substrate. The cultivated NA-MUG plate is exposure to the wavelength 366 nm ultraviolet (UV) light (6 W). The presence of bright blue fluorescence is considered a positive response for *Escherichia coli* (*E. coli*).

Detailed information on *Escherichia coli* determination is provided in Appendix D.

4.3.3 Determination of Intestinal *Enterococci*

The samples should be tested immediately after they are collected on board in accordance with the standards stipulated in water quality- detection and enumeration of the intestinal *enterococci* ISO 7899.2-2000.

Shake the water sample over 25 times at least before testing to make the water sample well mixed. Connect the sterilized filter device to the Buchner flask, put the membrane at the bottom of the filter with a germfree tweezer, and certain amount of water sample is sucked to the filter, and be sucked and filtered by the vacuum pump. After all the water sample liquid passes through the membrane, clean the edges of the filter with 20 mL to 30 mL normal saline for twice at least. Then, turn off the vacuum pump and turn on the filter, and take the filtered membrane by a germfree tweezer onto the surface of the Slanetz and Bartley medium (membrane intestinal *enterococci* culture medium). Ensure that no bubbles in the middle of the membrane and the medium. Put the plate upside down in temperature of 36 ± 2°C for 44 ± 4 h. After the culture time is over, all the colonies which is red, nut brown or pink, no matter in the middle or full over the plate are all typical. If there are typical colonies formed, transfer the membrane and the colonies using the germfree sweezer to the Bile–aesculin-azide Agar plate which has been preheated to 44°C, culturing for 2 h at 44 ± 0.5°C, then observe the plate, if the color of the culture medium around the colonies is brownish black, it means the colonies are positive, these colonies is counted as intestinal *enterococci* (note: counting when the colonies are uneven distributed or bulge will affect the identification of the positive colonies. The color will diffuse to the colonies nearby). Count the membranes which are proved to be intestinal *enterococci* colony, cfu/100 mL.

Detailed information on intestinal *enterococci* determination is provided in Appendix E.

4.3.4 Determination of *Vibrio cholerae*

According to “Diagnosis Standard for *Vibrio cholerae* (WS 289-2008)”, water samples are inoculated in basic peptone water medium. Place the enrichment medium with samples into incubator for enlargement cultivation at 37°C for 6-8 h. Then the strong and weak nutrient mediums are inoculated, they are used to isolated culture and made cultivated substance morphology observation. *Vibrio cholera* in different isolation mediums presents different characteristics. Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.

Detailed information on *Vibrio cholerae* determination is provided in Appendix F.

4.4 Temperature

Temperature is measured *in situ* using a calibrated thermometer. Temperature is reported in ^{o}C.

4.5 Salinity

Salinity is measured *in situ* using a calibrated salimeter. Salinity is reported in PSU.

4.6 Particulate Organic Carbon (POC)

POC is determined based on spectrophotometry method. According to "Chinese Gulf ecosystem observation method (2005)", carbon is wet oxidized by acidic dichromate; the decrease of the extinction value of the yellow dichromate solution may indicate the quantum of oxidized carbon.

Detailed information on POC determination is provided in Appendix G.

4.7 Total Suspended Solids (TSS)

The sample is filtered through a filter (0.45 μm) on board, and the filtered membrane is stored at 4^{o}C until analysis. TSS is measured at BWDL in accordance to National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.4) - Part 4: Seawater analysis.

Detailed information on TSS determination is provided in Appendix H.

5. QA/QC Checks

Four data quality indicators (DQIs) will be used to interpret the degree of acceptability or utility of the data obtained in the project. These are precision, operator bias, comparability and representativeness, and their protocols are described.

5.1 Precision

Precision refer to the measure of agreement among repeated measurements of the same property under identical or substantially similar conditions; calculated as either the range or as the standard deviation. It may also be expressed as a percentage of the mean of the measurements, such as relative range or relative standard deviation (coefficient of variation).

For samples of organisms ≥50 μm and ≥10 to <50 μm collected at the shipboard testing, within sample precision is measured by analyzing at least two slides from every sample collected. Precision is quantified by calculating the coefficient of variation (CV) among the subsamples analyzed for each sample using the following equation:

$$\%CV = \frac{SD}{mean} \times 100\%$$

Where, SD = standard deviation among subsamples; mean = mean live organism density among subsamples. The acceptable analytical precision is ≤30% for critical parameters.

With respect to samples involving water quality and bacteria collected at the shipboard testing, precision is evaluated by analyzing at least 10 percent of samples in duplicate and calculating the Relative Percent Difference (RPD) as determined by the following equation:

$$RPD = \frac{|x_1 - x_2|}{\frac{x_1 + x_2}{2}} \times 100\%$$

Where, x_1 = sample; x_2 = duplicate sample. The acceptable analytical precision is ≤20% for critical parameters.

5.2 Operator Bias

Bias refers to the systematic or persistent distortion of a measurement process that causes errors in one direction.

Operator bias relative to organisms ≥50 μm are evaluated for every treatment discharge sample collected at the shipboard testing. In this situation, one out of every ten slides analyzed by the primary taxonomist is also analyzed by a second, suitably-qualified zooplankton taxonomist. The duplicate analysis is conducted such that the second

operator does not know the results of the first operator's analysis. Additionally, one out of every ten influent or control discharge samples is analyzed by a second zooplankton taxonomist. In the event that there are less than ten total control samples collected during a treatment technology performance evaluation, at least one influent or discharge control sample is evaluated, with one slide analyzed in duplicate.

Operator bias relative to samples of organisms ≥10 to < 50 μm collected at the shipboard testing are evaluated using at least two treated discharge samples per set of three test trials and at least one influent or control discharge sample per set of three test trials. In this situation, for every sample analyzed by the primary taxonomist that requires evaluation, a second, suitably qualified taxonomist simultaneously analyzes the same sample. The analysis is conducted such that the second operator does not know the results of the primary operator's analysis, and vice versa.

BWDL evaluates operator bias for samples of bacteria by having a second, suitably-qualified operator count at least 10 percent of all experimental chambers. Analysis occurs immediately following analysis by the first operator and is carried out in a manner such that the second operator does not know the results of the first operator's analysis.

The BWDL performance measurement for these sample types is relative percent difference (RPD). The acceptable RPD is <20% for critical parameters.

5.3 Comparability

One control test run will be conducted for each of test cycle as a reference and to identify any changes in critical parameters not caused by the equipment itself.

5.4 Representativeness

Representativeness is a qualitative measure of the degree to which data accurately and precisely represents a characteristic of a population parameter at a sampling point or for a process condition or environmental condition. The representativeness will be achieved through (1) all samples will be taken in triplicates; (2) complete mixing will be ensured before any withdrawal of samples or *in-situ* measurement; (3) one control will be conducted for each of the test cycle to identify any changes in critical parameters not caused by the equipment itself.

6. Quality Management Plan

As the testing organization, the Ballast Water Detecting Lab of Shanghai Ocean University will carry out the project quality management in compliance with the Quality Management Plan (QMP) and takes part in the comparison of the testing results with those obtained by competent labs specialized in the same testing field, and participates in the proficiency testing program organized by authorized organization according to the lab file which is called the Procedures for Testing Result Quality Control.

Retest of the samples in the retention time, retest the same sample by the same method or different method, and retest the same sample using the same instrument or different instrument to assure the quality of the test result. Keep the sensitivity, accuracy, deviation allowance range, precision of the parameters; ensure the reliability and integrity of the data.

Enhance the quality awareness of the test personnel; make a clear division of quality responsibilities. The test undertaking organization should be supervised by the entrusted organization and the technical supervision organization. The test undertaking organization should take the quality control procedures in the test process into the quality operation system and make the quality plan to comply the quality system and requirements of the testing project.

6.1 Quality Management of Field Sampling

Assure the quality of field sampling and analysis. Prepare the procedures for conducting the sampling and avoid the samples being polluted. Keep away from the interference of the instruments. Select sampling facilities, sample bottles appropriate for the testing items. Take antipollution measures for accessories in testing place; minimize the influence of the interface concentration. The pretreatment of samples should be completed in situ right after the samples are collected, and then add some stabilizer and store in low temperature. Items which are susceptible for microbes' activities or change fast with time should be finished testing within the stipulated time.

Indicators like salinity, temperature should be measured in field. During the field test, turn on the instrument and leave it to be warmed-up until the instrument reading and the flow of the ballast water management system become stabilized, and then wash the sample bottle for two times with a little of water sample, afterwards, fill the bottle up with sample, the probe of instrument in the sample bottle, get the reading after the instrument is stabilized. When collecting the suspended solid water sample, wash the sample bottle for two times with a little of water sample, and then fill the sample bottle to the fully slowly. Ensure the water samples are stored in cool and shady place and transported to the lab. Ensure the water sample be filtered within 24 h. The POC samples collected simultaneous are divided in the lab; try to use the ground glass sample bottle to collect the samples in order to avoid the absorption of C of plastic products. Before use, all the glass containers should be immersed in the Sulfuric acid and potassium dichromate lotion for 24 - 28 h; then be rinsed with tap water and washed again with de-carbonized water, and the de-carbonized water should be

prepared in advance. When collection samples in situ, wash the sample bottle with a little of water sample first and then collect the samples and refrigerated transport the sample to the lab.

Collect 10 - 50 μm viable organisms sample by method of sediment and concentration counting of certain volume of water sample. There is no need to rinse the sample bottle when collect the samples, collect certain amount of well mixed water samples. Water sample of viable organisms greater than 50 μm is prepared by collecting the organisms filtered by a 50 μm screen organism net and count the total number. Ensure the organism net is clean and tried before collecting. Viable organism staining agent should be prepared temporarily (to avoid the failure of staining agent as time goes) before sampling. Add quantitative staining agent to the collected viable organism sample for enough time and then add formalin to fix the sample. Right after the viable organism samples are collected, transport them to the lab (protect them from light and vibration) and analysis as soon as possible. The microbe sampling and test personnel should be trained and be equipped with basic knowledge of microbes. When conduct the microbe testing, all the containers should be sterilized. Prepare a set of trip blank and field blank during each sampling process. One regent blank should be prepared in each batch of samples or ten samples. Keep record of all the original data of the initial dilution water samples for review. Each dilution degree of water sample should replicate.

6.2 Quality Management of Lab Sample Analysis

6.2.1 Quality management of chemical reagents

Chemical reagents used in lab sample analysis should be prepared to solutions in accordance with prescribed conditions. The solutions should be stored in right conditions and used within the prescribed period. The self-prepared solutions are allowed to use unless they are calibrated to be qualified with the guarantee value of national standard solution. The blank value of reagent should be in the same level with the analysis detection limits. If the value is too far over the detection limits, the causes need to be found. And main agents are purified which have great agent blank value or change the reagents (use a new batch number of agents or agents produced by other manufacturers). And all the regents should be checked before use. In the cases when the blank value is hard to be lowered, add appropriate amount of reagent. During analyzing, parallel test the analysis blank and monitor the variation of the blank value.

6.2.2 Quality management of containers

Make a clear understanding of requirements for the materials used in containers, select the right material. The characteristics of container material should have the least pollution to the water sample and be easy to clean. And it should be inertia to the chemical activity and biological activity to protect the water sample from reacting with the container to the maximum extent. The capacity of dealing with temperature fluctuation, resistance to rupture, sealing property, capacity of reopening, volume, shape, mass and possibility for reuse of the sample storage containers should be taken consideration when selecting the containers.

For most samples which include inorganic compositions, containers which are made of polyethylene, polytetrafluoroethylene or eater polymer are chosen to use; for the storage of samples for determining and analyzing the conductivity and pH in water, containers which are made of high density polyethylene are used; for the storage of organic chemical and organism samples, glass containers are used. The containers should be cleaned in the right way; the compositions of the detergent should not include the substance to be tested. The new container should be cleaned thoroughly; the substances to be tested determine which detergent to choose.

For general use, taps water and detergent are used to clean dust and packaging matter, then immersed in the chromic acid and sulfuric acid detergent, and at last rinsed with stilled water. For those used containers, there are usually grease, heavy metal and residents in the bottom and wall of the container, there once they are reused, and they must be cleaned before being used. For those glass containers with stoppers, the ground part is often with digestions and absorptions. Polyethylene is susceptible to absorb oil or grease, heavy metal, sediments and organisms and it is hard to clean. So, much attention should be paid when cleaning the containers made of polyethylene. Before the container made of polyethylene is used, clean with 1 mol/L hydrochloric acid solution and immersed in the (1+3) nitric acid solution for a long time. Before the sample bottle used for storage and environmental parameters analysis is used, clean it with nitric acid solution, and then rinse with stilled water to remove the heavy metal and chromate residual. If the organic composition to be determined is tested after extraction, the glass bottle may be cleaned with extraction detergent.

6.2.3 Quality management of instrument

The analysis instrument for testing should be in compliance with the stipulations of the Specification for Ocean Monitor GB/T 17378. Instruments are checked and calibrated by specified personnel in regular times. The instruments should be cleaned with stilled water after being used and immersed in the protection liquid to avoid the residual of samples and corrosion of the instruments. Or maintain the instruments according to the instrument operation manual to keep off of measurement error next time, and conduct the instrument interval check as necessary.

6.2.4 Quality management of environmental parameters

After POC samples are collected, use fiber glass membrane with 47 mm diameter and 0.45 μm diameter and the standard micropore filter to treat the samples. The membrane must be burned in the 450-500^{o}C muffle for 24 h wrapped in aluminium foil to remove the oxidizing substances (the burning temperature should not exceed 500^{o}C, or else, the filtration characterization of the membrane will change).

When the TSS is filtered, clip the membrane with a stainless steel tweezer for fear of pollution. Prevent the seawater from flowing backward and then damage the vacuum pump. And drain out the wastewater in time. Keep the ambient tidy when drying the sample.

6.2.5 Quality management of lab test methods

The lab can undertake the test task on condition that it is accredited the Metrology certificate. Test method is selected mainly based on the precision, accuracy and detection limits of method, to give due consideration of factors such as cost, instrument condition and test cycles and the skill level of personnel. The test methods used should be verified by standard novelty search.

7. Instrument/Equipment Testing, Inspection and Maintenance

The instrument manager is responsible for compiling the Check List of the instruments, and establishes the file of instruments and identifies the instruments with related labels. The operator of instruments should be an authorized staff or one with vocational test staff certificate. All operators should be approved by the lab to carry out the operation. Use of equipment should be strictly in accordance with operating procedures. The staff is asked to operate the instrument as trained to be in order not to get the invalid test result. The user of the instruments should check the status an environment condition of the instrument (including whether it is in valid period, need maintenance or not, if stabilized or not) before and after use. And fill the Use Record of the Instrument.

If there is abnormal phenomenon (overloading, wrong operation, questionable result displayed) for the equipment, the user should stop the operation and stick a red mark on it. Separate the abnormal instrument to avoid misuse. If the instrument falls out the direct control of the lab for example: removed to other places, sent for repair or calibration, after the instrument is back, the instrument attendant should check the function and the calibration status of the instrument and recovered to use until the results displayed are satisfying. The instrument manager takes charge of checking the instruments to prevent the instruments from damaging and losing. Make an inventory of the instruments annually. If there is damage or lose of instrument, repair or handle in accordance with the Control Procedures for Nonconsistant Test Work.

A specified worker is appointed for the maintenance of the instrument in use. Power on once per month at least (1 - 2 h) to check if the instrument is normal and keep record. The instrument manager is responsible for organizing the instrument user to make the routine maintenance plan, and to form the Routine Maintenance Table of Instruments. The instrument user makes the maintenance of the instrument to comply with the items and periodic times in the table and keep the record meanwhile.

8. Instrument/Equipment Calibration and Frequency

Instruments need to be verified and calibrated by legal metrology verification service agency. The instruments are delivered by the lab synthesizer according to the Quantity Traceability Procedures, aiming to get the qualified certificate. The instrument attendant performs the periodic calibration of the instrument. If the correction factors are obtained after instrument calibration, the instrument attendant is responsible for updating of all the backups and the correcting of related data. The frequency for calibration is once per 12 months, once per 6 months for special instrument. Refer to Table 12 for the calibration requirements of the instruments that will be used in the test.

Table 12 Calibration Requirements of the Testing Instruments

No.	Parameters	Equipment Name	Model	Measuring range	J expand	Frequency of Verification Calibration Requirement
1	Heterotrophic bacteria	GZX-Ⅲ serial light incubator	GZX-400B S-Ⅲ	(0~60)°C	U=0.3°C, (k=2) ± 0.2°C	Annually
2	*Escherichia coli*	GZX-Ⅲ serial light incubator	GZX-400B S-Ⅲ	(0~60)°C	U=0.3°C, (k=2) ± 0.2°C	Annually
3	*Vibrio cholerae*	GZX-Ⅲ serial light incubator	GZX-400B S-Ⅲ	(0~60)°C	U=0.3°C, (k=2) ± 0.2°C	Annually
4	Intestinal *enterococci*	LDZX model vertical pressure steam sterilizing pot	LDZX-75 KBS	(50~126)°C	U=0.5°C, (k=2) ± 0.2°C	Annually
		Model DK electric-heated constant temperature water bath kettle	DK-S26	(RT+5~99)°C	U=0.3°C, (k=2) ± 0.3°C	Annually
5	Phytoplankton (10-50 μm)	optical microscope	Leica DM2500	(40~1000)x	±5%	Annually
6	Zooplankton (≥50 μm)	optical microscope	Leica DM2500	(40~1000)x	±5%	Annually
		stereomicros-cope	Leica S8	(10~80)x	±5%	Annually
7	TSS	electronic balance	AL104/01	(0.0001~110) g	I grade	Annually
8	Temperature	thermometer	(0~40)°C	(0~40)°C/0.2°C	0.62°C	Annually
9	POC	ultra violet spectrophotometer	UV-2000 model	(190~2600) nm	IV grade	Annually
10	Salinity	salimeter	YSI85-25	0~80 PSU	±2%	Annually

9. Data Collection Requirements

The technical director summarizes the results obtained both from filed work and lab test for shipboard test and organizes the data acquisition and statistics. Prior to data statistics, the test personnel should check the test data first. Check if the original data is integrated and if it meets the requirement, if the calculation and conversion of data is right. Mutual correction is preferred by test personnel after the test data is checked by the test personnel. The reviewer should carry out the review in conformance with the standards, procedures, norms and enforcement rules, and if calculation is required, the calculation for mulas and the calculation process should be checked. Check whether the calculations, the rounding off and the conversion are right. The reviewer should review the original data thoroughly at the time of checking the test reports for the reliability and the matching of the data. The data verified to be right is collected and summarized by the technical director.

10. Data Management

The sampling data and data determined in field should all be record in waterproof table or to create the electronic document right after the samples are taken in field. The management of the electronic data is in accordance with the Procedures for Computer Management.

10.1 Data Record

To make the record meet the standard and ensure that sufficient information is collected, the lab adopts the uniformed and approved form of record table. The sampling records should be prepared with pen or ball-pen. The handwriting should be clear. And the record should be verified and integrated. There should be date of record, signature of recorded person and the record number on the record, and the technical record should include the signatures of test personnel and reviewers. The technical record should include technical parameters. All the technical parameters, data, observation results and calculations should be kept being recorded in time, with no replenish.

If the sampling records need to be modified, two lines should be written on the original records, which should be made out. Then the modified records should be written on the blank on the top right of the original records with the mender's stamper or signature or abbreviative signature. All the records should be collected, filed and preserved.

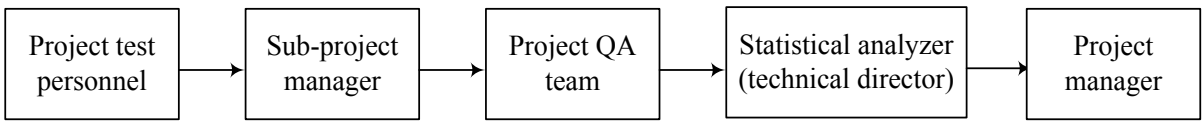
10.2 Data Confirmation

The test personnel conduct the test in accordance with the requirements and standards related. The quality supervisor takes necessary actions to oversight the test. After the test is finished, the technical director need to sign for confirmation.

10.3 Data Conversion

The quantity of the plankton is counted and converted to the uniform unit required by the project by statistical analysis. Therefore, organism samples with particle sizes between 10 μm and 50 μm are converted into cells/mL; particle sizes greater than 50 μm are converted to cells/m^3; the bacteria samples are converted to cfu/100 mL.

10.4 Data Delivery



10.5 Data Analysis

The original data record is collected by related test personnel, and the test personnel will calculate the final test results according to the conversion method of the parameters

and the corresponding curve. The calculation process of data is included in the original record. Take the assessment for the uncertainty occurs in the testing process based on the stipulations in the standards for testing all parameter and determine the significant digits for values. At last, the technical director analyzes the removal rate of organisms with different particulate diameters at each steps of ballast water management, and calculates the removal effect. The rounding off method of specific values is as follows:

1) Refer to *The Rule of Data Revising* (GB 8170-87) for rounding off the values

The rule for rounding off of the numerical values is a round 5 (the digit of the tested valid number is determined): when the rounding off number of the measured value is less or equal to 4, then rounding down; if the rounding off number in the measured value is more than or equal to 6, then rounding up; if the rounding off number in the measured value is equal to 5, rounding up if the mantissa rounded up number is even number, and rounding down if the mantissa rounded up number is odd number. The measured values are rounding off by this way.

In calculating and reading of data, the digits of data might be more than prescribed, for example, the digits of data calculated in calculator may be 7, and when weighed on the analytical balance, only 5 digits of data is obtained, so it is necessary to rounding off the redundant digits. The process for cutting the redundant digits or digit is called the rounding off process, and it is in accordance with the rules of Four Rounding Down and Five Rounding up.

2) Data calculation rules

The data calculation rules are determined by the law of error transmission.

Plus-minus method: transmission of the absolute errors of the measured values. The absolute error of the max absolute error of measured values determines the uncertainty of the analysis result. Therefore, the retention of the significant digit of the summed value of several measured values should base on the number which has the least digits after the decimal point.

Multiply-division method: transmission of the relative errors of the measured values. The relative error of the result should be in accommodation with the value with the max relative error. Therefore, rounding off of the values should be in accordance with the least significant digits.

Scale values of the volumetric containers used for titrimetric analysis (burette, volumetric flask, and pipette) are all with four significant digits. So the number of significant digits of the test data result is four.

3) Formula for calculating removal rate

Removal rate = (density before treated – density after treated) × 100%/density before treated

10.6 Data Storage and Retrieval

All the test data record should be kept by the data administrator. The preservation time of the copies of the original test record, test reports is five years and the data administrator takes charge of the safe custody of the files and records. The records should not be let out or loaned to people unrelated and the customer's business secret should be kept.

The internal staff should go through procedures for loan or copy of the documents, and he or she should fill in the registration table. For external staff who wants to loan or retrieve the records, he or she should be approved by technical director, after the technical director give approval, he or she can go through the loan procedures and fill in the registration table. Read on site, no taking away. The user or keeper of the records should comply with the procedures for keeping the secret and proprietary of custom, do not copy without permission and forbid revealing.

11. Evaluation and Supervision

11.1 Evaluation and Response Actions

Project supervisor of the shipboard BWMS testing project is responsible for conducting a continuous improvement actions based on the quality policy, quality objectives, approved result, data analysis, data correction, preventive measures and management review including analyzing and assessing the status; looking for and finding the aspects needed to be improved (looking for the improvement opportunity); ensuring improvement aims; establishing improvement scheme and reviewing the scheme, then selecting the optimal one; implementing the responsibilities and related resources, and putting forward the improvement scheme; monitoring and measuring the implementation situation to make sure whether the it is effectively implemented; formally taking the effective measures; the corrective and preventive actions should be taken into the plan and the management of daily improvement activities.

To determine the causes of discrepancies and look for the improvement chance by way of internal approval, management review, custom feedback, ability verification or other way of data analysis of quality control result. If preventive measures are taken, supervise and monitor the implementation of them, to minimize the possibility of nonconformities and look for improvement chance. Conduct the assessment in accordance with the lab's improvement control procedures, correction measures procedures, preventive measures procedures, test result quality control procedures and the management review control procedures and take the emergency response measures.

11.2 Test Report

The technical director of the testing organization submits the test reports, and the quality manager of the testing organization submits the uncertainty report to the quality management team of shipboard test for ballast water management system. The supervisor of the testing organization briefly summarizes the results of related parameters and proposes a new project quality assurance plan to the supervisor of the entrusted organization for summary and renewal. The test reports of each item and the test results should be precise, clear, and objective and conducted in compliance with the test methodology.

Each test reports should include at least information as follows: test designing, identification of the methodology, status description of the tested material and cleared label, the acceptance date and the test date, the test result, the test report approver or equivalent mark; if the test result need to be explained, there should be announcement about the test method deviations and evaluation uncertainty included in the test report. In the cases when the testing results provided by a subcontract party are included in the testing report, those results should be marked clearly. The subcontract party should report the results in the way of paper edition or electronic edition.

12. Data Validity and Usability

12.1 Data Review, Verification and Validation

Check and review all the data from field determination and lab test and verify the integrity, continuity, validity of the data, and check whether the items meet the requirements. When the data results are in consistent with the quality control data of ballast water management system and the data quality achieves the objectives of this project, then the ballast water management system is acceptable.

12.2 Verification and Validation Methods

The review, verification and validation of date should be performed to ensure the data meets the criteria. The verification and validation methods include self-assessment, taking part in the reconciliation activities with other labs organized by authorized parties and the ability verification plan. The authorized signatory verifies the quality control data by statistical technology annually and makes the verification reports, then input the management review. If the quality control results are not satisfying or unstable, look for the causes for problems and take actions in accordance with the lab's Corrective Measures Procedures, Discrepancy Test Work Control Procedures, and Preventive Measures Procedures.

The data validation includes all the task plans of the ballast water management system test except the data verification confirmation, including the quality control result assessment for determination of field sampling data, assessment for determination of lab parameters, discrepancy analysis of sample storage and pretreated, the sample test limitation time range verification, the traceability of methodology for test reagents and test standards, verification of the analysis sensitivity in conformance with QAPP, deviation analysis of sampling and analysis with requirements of QAPP, the verification of calculated results, to ensure that QAPP includes relevant information on all the parameters and samples.

12.3 Reconciliation with Test Data Quality Objectives

Data generated in this project is analyzed and reconciliated with the data quality and project requirements in accordance with the guidelines for approval of ballast water management systems (G8) and discharge requirements of ballast water (D-2 regulation). The data meet the requirements of the project and the D-2 standard, and achieve the treatment effectiveness of ballast water management systems and the data related documents will be applied to the authorized organization as appropriate.

13. References

IMO (2004). International Convention for the Control and Management of Ships Ballast Water and Sediments. As adopted by consensus at a Diplomatic Conference at IMO, London, England, February 13 2004.

IMO (2008). Guidelines for Approval of Ballast Water Management Systems (G8), Resolution MEPC.174 (58). Adopted on 10 October 2008.

United States Environmental Protection Agency (2010). *Environmental Technology Verification Program (ETV) Generic Protocol for the Verification of Ballast Water Treatment Technology,* Version 5.1. Report number EPA/600/R-10/146.

GB 17378.7 (2007). The National Standard of the Specification for Marine Monitoring-Part 7: Ecological Survey for Offshore Pollution and Biological Monitoring.

GB/T 5750.12 (2006). Standard Examination Methods for Drinking Water-Microbiological parameters.

ISO 7899.2 (2000).Water quality- detection and enumeration of the intestinal *enterococci*-Part 2: Filter membrane method.

GB 17378.4 (2007). The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis.

Chinese Gulf Ecosystem Observation Method (2005). China Environmental Science Press.

Diagnostic Criteria for Cholera. WS 289-2008.

14. Appendices

Appendix A Standard Operating Procedure for Organisms ≥50μm Sample Analysis

1. SCOPE AND APPLICATION

This method is utilized to identify and enumerate organisms ≥ 50μm in ballast water samples. Here we describe a method using a Janus green B staining solution to tag viable cells.

2. METHOD OVERVIEW

Organisms will be enumerated under dissecting microscopes (nominally 10x-80x) to determine concentrations of live and dead organisms ≥50μm in minimum dimension. The samples will be collected in zooplankton nets constructed of mesh with an open square 35μm on each side (50μm on diagonal). The method involves microscopic examination of the living or dead organisms ≥50μm in minimum dimension by the staining degree of staining solution, including zooplankton and phytoplankton.

3. SAMPLE COLLECTION AND TRANSPORTATION

Samples for organisms ≥ 50μm in minimum dimension will be collected in custom constructed zooplankton nets. The nets are built by Institute of Hydrobiology, Chinese Academy of Sciences to our specifications. The mesh size will be 35μm nylon wire material, with all seams double stitched, the diagonal of the mesh size will be 50μm. The description of sample collection is fully elaborated elsewhere.

The zooplankton nets will be rinsed with filtered sample water at the end of each concentrating operation to ensure all organisms are collected in the cod-end. The net contents will be transferred to clean, wide-mouth glass jars to a final volume of nominally 50 mL (measured volumetrically).

4. MATERIALS, INSTRUMENTS AND REAGENTS

- Quantitative liquid pipette
- 4% buffered formalin
- Stereoscopic microscope
- Dissecting needle
- Sedge wick Rafter counting chamber, 1 mL
- Janus Green B solution

5. ANALYTICAL PROCEDURE

After adding Janus Green B solution and formalin, place the sample 24 h without

disturbance. We then concentrated the samples to 50mL. Subside the sample for at least 12 hours, and then absorb 1 mL sediment onto a Sedge wick Rafter counting chamber. Numbers of living and dead individuals are counted at 80x magnification using microscopes with bright field (white light) illumination. We judge survival or death of the organisms by observing the color of cells, and once cell is damaged, the absorbing neutral red may disappear. At last, record the number of living cells with Janus Green B.

After finishing counting all the subsamples, supply 4% buffered formalin to store and carefully identified the species for organisms ≥ 50μm in lab.

6. REFERENCES

Huang Zong-guo. Marine Species and their distribution in China. Beijing: Maritime Press, 2008.
National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.7) - Part 7: Ecological survey for offshore pollution and biological monitoring.

Organisms ≥ 50μm analysis log sheet

The project name			
Test date		Sampling site	
Sample number		Sample State	
Sampling time		The volume of filtered water	
The volume after		The volume of	
Starting time of testing		Ending time of	
Detection conditions	Temperature： ℃， Humidity： %RH		
Instrument	light microscope（SHOU-BWEQ-02）、anatomize lens（SHOU-BWEQ-04）		
Testing standards	GB17378.7-2007, Standard Operating Procedure for Organisms ≥50μm Sample Analysis		
Testing results（number of the living individuals）			
Density of living organisms（ind./m^3）			
Note：Density of living organisms= number of the living individuals / the volume of filtered water			

Analyst: Assessor: Approver:

Appendix B Standard Operating Procedure for Organisms ≥10 μm to <50 μm Sample Analysis

1. SCOPE AND APPLICATION

This method is suitable for analyzing viable phytoplankton less than 50 micrometres in maximum dimension and greater than or equal to 10 micrometres in minimum dimension. The sampling, pretreatment of samples and analyzing was described. The maximum dimension on the smallest axis without thorn, antennae and flagella is ≥10μm and <50μm.

Here we describe a method using an algae staining solution to tag viable cells. Estimate the living or dead cell by the staining degree of staining solution. Staining solution can be absorbed by living cells. Once cell is damaged, the absorbing staining solution may disappear. In this way, we can judge the living or dead cells.

2. SAMPLE COLLECTION, PROCESSING AND PRESERVATION

2.1 **Sample collection**

According to G8 guideline of the IMO, 3 × 10 L samples of influent water and discharge control water, 3 × 3 × 10 L samples of discharge treated water should be sampled for analyzing 10 - 50μm organism.

2.2 **Sample processing**

The sampled water is slowly sieved through plankton net (10μm diagonal dimensions). The remained organism on the net is washed by filtered water for at least three times. Collect the organisms in a 60 mL bottle. Add 3 drops of staining solution. After 15 min, add 2 mL formalin to the concentrated sample.

2.3 **Sample preservation**

After subsampled and analyzed, we add formalin to the concentrated sample. Samples with formalin can be preserved for three to six months.

3. APPARATUS

Microscope (Leica DM 2500; 100x, 200x, 400x)
Plankton counting chamber (0.1 mL)
Pipette (0.1 mL)

4. REAGENTS

Neutral red staining solution (should be stored in dark, at -20℃ for one year)
Formaldehyde solution (Formalin, 37 - 40%)

5. ANALYTICAL PROCEDURE

After adding neutral red and formalin, place the sample 24 h without disturbance. We then concentrated the samples to 50mL. Stained subsamples are shaken up and down, and then absorb 0.1 mL onto a plankton counting chamber with cover glass. Numbers of living and dead cells are counted at 400x magnification using microscopes with bright field (white light) illumination. We judge survival or death of the organisms by observing the color of cells, and once cell is damaged, the absorbing neutral red may disappear. At last, record the number of living cells with neutral red.

6. REFERENCES

National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.7) - Part 7: Ecological survey for offshore pollution and biological monitoring.

Organisms ≥ 10μm and <50μm analysis log sheet

The project name		Project number	
Date of entrustment		Test date	
Sample number		Sample State	
Testing standards	GB17378.7-2007；Standard Operation Procedure of testing		
Detection conditions	Temperature： ℃，Humidity： %RH		
Instrument	Fluorescence microscopy、plankton counting chamber		
Sample receiving time		The volume after filtered mL(V_1)	
Sample volume L（V）		The start and end time of testing	

Testing Results					
First	Testing Volume(V2)：	Second	Testing Volume (V2)：	Third	Testing Volume (V2)：
Number of living organisms（cells）X		Number of living organisms（cells）X		Number of living organisms（cells）X	
Density（cells/ml）D_1		Density（cells/ml）D_2		Density（cells/ml）D_3	
Average density（cells/ml）D^1					

Note：Density（D）=$X*V_1/(1000V*V_2)$

Average density（D^1）=$(D_1+D_2+D_3)/3$

Analyst: Assessor: Approver:

Appendix C Standard Operating Procedure for heterotrophic bacteria Analysis

1. Method Overview

Plate count method is one of the most simple and effective methods testing the total number of heterotrophic bacteria. The test result of this method is very intuitive, which can get results with macroscopic observation of visible colony on the petri plate. We choose 2216E agar plate test salt samples, and the nutrient agar plate used in the detection of salt-free samples.

The single bacteria cultivated on plate nutrient medium could form the visible colonies. One colony represents one cell. Relying on counting the number of visible colonies, the plate count method could get the number of bacteria.

The keys to count bacteria are: to separate bacteria on sample to single cell as far as possible; prepare diluents of different concentrations and evenly inoculate certain volume of the diluents on petri plate with solid nutrient medium (hereinafter to be referred as plate).

2. Method References

- The National Standard of the Specification for Marine Monitoring—Part 7: Ecological Survey for Offshore Pollution and Biological Monitoring (GB 17378.7-2007/10.1).
- Standard Examination Methods for Drinking Water-Microbiological parameters (GB/T 5750.12-2006/1.1).

3. Instruments and Reagents

- Tween-80 solution: mix 1 mL Tween-80 with 2000 mL distilled water.
- 2216E nutrient medium: The components of 2216E nutrient medium: 5 g peptone, 1 g yeast extract, 0.1 g ferric phosphate, 20 g agar; 1000 mL aged seawater.
  Preparation: heat up above substances for dissolution and use NaOH solution to adjust pH value to 7.6. Put the solution into conical flask and place them into autoclave sterilizer for disinfection under the condition of 121°C (about 105 kpa) for 20 min. After that, pour nutrient medium into the disinfected plate and the volume of nutrient medium in every plate is about 15 mL. When the samples cooled and solidified, put them into refrigerator for perseveration.
- Nutrient-Agar-Medium: The components of nutrient agar medium: 10 g peptone, 3 g beef extract, 5 g NaCl , 10 - 20 g agar, 1000 mL aged seawater.
  Preparation: heat up above substances for dissolution and adjust pH value to 7.4 - 7.6. Put the solution into conical flask and place them into autoclave sterilizer for disinfection under the condition of 121°C (about 105 kpa) for 20 min. After that, keep the medium at 50°C until used.
- Constant temperature incubator
- Autoclave sterilizer

- Petri dish: diameter is 9 cm
- Pipet: 1 mL
- Wild-mouth sampling bottle:1000 mL
- Conical flask
- pH meter
- Super clean bench
- Common used instruments and equipment in laboratory

4. Analytical Procedure

- Add 1 mL Tween-80 solution to per 100 mL water sample. Shake well to separate bacteria in sample to single cell.
- 1 mL water sample is used to preparing the 10 times diluted water sample. And then mix the 10 times diluted water sample thoroughly. Following the same procedure, continuous dilution should be done until getting the required extent which depends on the amount of bacteria in water sample. The proper bacterial colony in every plate should in range of 30 - 300 cells. Each dilution extent should have three duplicate plates.
- After inoculating the samples on plates, place the plates upward for at least 20 - 30 min.
- Place the plates into the 25°C constant temperature incubator for 7 days' cultivation. If the samples were fresh water, place the plates into the 37°C constant temperature incubator for 2 days' cultivation.
- Thereafter, take the plate out to count bacterial colony.

5. Data Analysis and Calculations

- Bacterial colony count method
  —If there is large lawn on the plate, the count is invalid. Select the plate which has the total number of colonies between 30 and 300. To obtain results, multiply the average number of colonies per plate by the dilution extent.
  — If the average numbers of colonies in two dilution factors are in the range of 30-300 colonies per plate, the count is determined by the ratio of two numbers. If the ratio is less than 2, the count takes the average number; if the ratio is greater than 2, the count takes the fewer colony number.
  — If plates from all dilutions have more than 300 colonies, compute the count by the multiplying the average colony number of the least dilution factor plate (the highest concentration plate) by the dilution factor.
  — If plates from all dilutions have less than 30 colonies, compute the count by the multiplying the average colony number of the highest dilution factor plate (the least concentration plate) by the dilution factor.
  — No colonies – if plates from all dilutions of any sample have no colonies and found no contamination, report the count as less than one times the dilution factor. For example, if the minimum dilution factor is 1:100, report the count as less than 100.
- Results will be recorded in the 'Total heterotrophic bacteria detection log sheet'.

ATTENTION

The following attentions should be noticed:

The bacteriological testing should strictly follow the sterile operation. The collected sample should be tested in time. The allowed storage time is no more than 4 hours. Otherwise, the sample must be stored in ice box, but the storage time is not more than 6 hours. Plates should be prepared in advance, or else the water on the fresh plate would influence the testing results.

Total heterotrophic bacteria detection log sheet

Sample No.	Time		The number of colonies					The density of total heterotrophic bacteria (cfu/mL)
	Sampling time	Detection time	The volume of sub-sample: 0.1 mL					
			0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Remark:								

Analyst: Assessor: Approver:

Appendix D Standard Operating Procedure for *Escherichia coli* Analysis

1. Method Overview

This SOP describes the testing method of *Escherichia coli* in water. With diameter of 0.45 microns of microporous membrane filter to filter samples, place the membrane on the Fuchsin Basic Sodium Sulfite Agar and cultivate at 37°C for 24 h, the selective medium were used to form the characteristic colony which is aerobic and facultative anaerobic gram-negative no-sporeforming bacillus.

The membrane of positive total coliforms was cultivated in medium containing fluorescent substrate(NA-MUG), which can produce β-glucuronidase that release fluorescent products because of decomposition of fluorescent substrate. The cultivated NA-MUG plate is exposure to the wavelength 366 nm ultraviolet (UV) light (6 W). The presence of bright blue fluorescence is considered a positive response for *Escherichia coli* (*E. coli*).

2. Method References

- The specification for marine monitoring-Part 7: Ecological survey for offshore pollution and biological monitoring (GB 17378.7-2007/9.2).
- Standard examination methods for drinking water-Microbiological parameters (GB/T 5750.12-2006/2.2, 4.2).

3. Instruments and Reagents

- Fuchsin Basic Sodium Sulfite Agar
- MUG nutrient agar medium (NA-MUG)
- Filter membrane 0.45μm
- The suction filter equipment
- Dressing forceps
- Other instruments are same as multi-tube fermentation
- Ultraviolet lamp: 366 nm, 6 W. Used to measure fluorescence reaction

4. Analytical Procedure

Start the examination preferably immediately after taking the samples. If the samples are kept at ambient temperatures, the examination shall begin within 6 h after taking the sample. Under exceptional circumstances, it is permissible for the samples to be kept at 5 ± 3°C for up to 24 h prior to examination.

- Filter samples

  10 mL water sample is used to preparing the 10 times diluted water sample. And then shake the sample well. Following the same procedure, the samples with different dilution factors such as 100 and 1000 can be obtained. Filter 100 mL

original sample, 10^{-1}, 100^{-2}, 10^{-3} dilution water samples, in respectively.
Treatment water: low-concentration samples. Filter 100 mL original sample directly.
To use sterile forceps clip sterilization filtration membrane edges, to face the rough. Stick on the filter bed has been sterilized, fix filter, 100 mL Water (As more water samples containing bacteria, can decrease to filter water samples, or samples will be diluted) injection in the filter, Open the valve of filter, filter under 5.07×10^4 Pa (minus 0.5 atm press).

- Cultivate: After filter the water, extraction of about 5 s again, turn off the filter valve, remove the filter. To use sterile forceps clip sterilization filtration membrane edges. Move on the fuchsine sodium sulfite medium, membrane filter intercept oriented on bacteria. Membrane filter should be totally against with medium, there shall be not bubbles .Then inversion of culture dish, put in 37°C incubator to cultivate for 24 ± 2 h.

- Pick comply with the following characteristics of the colonies gram staining and microscopy:
  Amaranth, colony with metallic luster;
  Crimson, colony without or with metallic luster;
  Pink, center color deeper colony.

- If the gram present negative bacillus coli, Lactose reino culation protein nutrient solution of arteries and veins, in 37°C cultivate 24 h. If produce acid gas, so the total coliforms are negative.

- Inoculation: Filter membranes with typical colony above were transferred to NA-MUG plate for *E. coli* detection under the sterile condition. Cultivate and section of bacteria faces up.

- Cultivate: Inoculated NA-MUG plates were cultivated in 36 ± 1°C for 4 h.

- Observation of result: The cultivated NA-MUG plate is exposure to the wavelength 366 nm ultraviolet (UV) light (6 W). The presence of bright blue fluorescence on the colony edge or the back of the colony is considered a positive response for *E. coli*.

5. Data Analysis and Calculations

Calculate the total number of *E. coli* that can produce blue fluorescent with formula as follows，report the total number of coliform bacteria in 100 mL water samples (cfu/100 mL).

Escherichia coli **detection and enumeration log sheet**

Sample No.	Time		Dilution rate				The number of Fluorescent validation	*E. coli* (cfu/100 mL)
	Sampling time	Detection time	0	10^{-1}	10^{-2}	10^{-3}		
The volume of filtered water (mL):								
Blank:								
Remarks:								

Analyst: Assessor: Approver:

Appendix E Standard Operating Procedure for Intestinal *Enterococci* Analysis

1. Method Overview

Membrane filter method is used to verify and count the enterococcus in the water. Amounts of water were filtrated by using the 0.45 μm membrane filter which can prevent the bacteria. The filter is placed on a solid selective medium containing sodium azide (to suppress the growth of Gram-negative bacteria) and 2, 3, 5-triphenyltetrazolium chloride, a colourless dye, that is reduced to red formazan by intestinal enterococci.

2. Method References

Water quality: Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method (ISO 7899.2-2000).

3. Instruments and Reagents

- Distilled water
- Slanetz and Bartley medium
- Bile-aesculin-azide agar
- Except for disposable glassware
- Sterile membrane filters, with a nominal pore size of 0.45 μm.
- Vacuum pump
- Incubator (capable of being maintained at 36 ± 2°C; 44 ± 0.5°C and 121 ± 3°C)
- Sterile forceps

4. Analytical Procedure

- Preparation of the sample: Start the examination preferably immediately after taking the samples. If the samples are kept at ambient temperatures, the examination shall begin within 6 h after taking the sample. Under exceptional circumstances, it is permissible for the samples to be kept at 5 ± 3°C for up to 24 h prior to examination.

- Filtration and incubation: Filter 100 mL water being examined. The collected sample should be transported at low temperature to laboratory immediately. Shake water sample vigorously 25 times before testing so as to make sample mixed well. Connect the sterile filter device to filter flask. Use the sterile tweezers to put filter membrane on the bottom of filter. Pipette some water sample into filter and open vacuum pump to start extraction filtration. After all the water passes through filter membrane, use 20 - 30 mL of sterile normal saline to wish the edge of filter at least two times, and then turn off the vacuum pump. Open the filter and use sterile tweezers to move the filter membrane to the surface of Slanetz and Bartley medium.

Prevent bubbles production between filter membrane and medium. Incubate the plates at 36 ± 2°C for 44 ± 4 h.

- Confirmation and enumeration: After incubation, consider all raised colonies which show a red, maroon or pink color, either in the center or throughout the colony, as typical. If there are typical colonies, transfer the membrane and the colonies, with sterile forceps without inverting it, onto a plate of bile-aesculin-azide agar which has been preheated to 44°C. Incubate at 44 ± 0.5°C for 2 h.

5. Data Analysis and Calculations

- Read the plate immediately after incubate at 44 ± 0.5°C for 2 h. The presence of brownish black around colonies is considered a positive response for enterococcus. Count the identified enterococcus and report the result as cfu/100 mL.

Record cfu/100 mL scores in intestinal *enterococci* detection and enumeration log sheet.

Intestinal *enterococci* detection and enumeration log sheet

Sample No.	Time		The volume of filtered water (mL)	The number of colony forming unit after 44h cultured at 36°C	The number of colony forming unit after 2 h cultured at 44°C
	Sampling time	Detection time			
Remarks:					

Analyst: Assessor: Approver:

Appendix F Standard Operating Procedure for *Vibrio cholerae* Analysis

1. Scope and Application

This method is utilized to detect *Vibrio Cholera* of serogroups O1 and O139 in ballast water.

2. Method Overview

Water sample be inoculated in basic peptone water medium. Place the enrichment medium with samples into incubator for enlargement cultivation at 37°C for 6-8 h. Then the strong and weak nutrient mediums were inoculated, they were used to isolated culture and made cultivated substance morphology observation. Vibrio cholera in different isolation mediums presents different characteristics. Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.

3. Sample Collection and Transportation

For isolation and detection of a higher probability *V. cholerae* from the samples, we choose concentrated water samples included plankton samples and water samples.

Water collection bottles should be cleaned with detergent, however the latter must not leave residue, should not be anti-bacterial and they should be pre-sterilized in an autoclave for 15 to 20 minutes at 121°C prior to use. Polypropylene bottles should be used for water samples. A sufficient volume of water and plankton should be collected to insure that appropriate analyses can be performed.

Processing of samples should begin soon after collection (typically within 24 hours of collection). If processing is delayed, the sample should be stored in a cool box at a temperature of 10 to 15°C until processing begins (not to exceed 8 hr). Based on type of examination, samples may require treatments; such as addition of direct viable count (DVC) reagents, before proceeding with further examination and testing. It is recommended that basic physiochemical parameters, e.g., temperature, salinity, pH, dissolved oxygen and conductivity of the water be measured on site at the time of collection as it is known that *V. cholerae* densities can be influenced by such parameters. These parameters can be measured on site.

4. Materials, Instruments and Reagents

- Non-selective enrichment medium -Basic peptone water medium
- No. 4 agar
- Alkaline nutrition agar
- N,N-Dimethyl-p-phenylenediamine dihydrochloride or
  1% N,N-Dimethyl-p-phenylenediamine dihydrochloride
- Vibrio cholera O1 in serum
- Vibrio cholera O139 in serum

- Filter paper

5. Analytical Procedure

- The water sample shall be immediately vaccinated in enrichment medium after sampling. In general, we absorb 50 mL water sample into 450 mL enrichment medium. Then place the medium into incubator for enlargement cultivation at 37°C for 6 - 8 h. Put one sterile inoculation loop under the biofilm of enrichment medium. Streak on a strong nutrient medium (No. 4 agar) and a weak nutrient medium (alkaline nutrition agar), respectively, and then incubate the two plates at 37°C for 18 - 24 h. Vibrio cholera in selective medium presents different characteristics.

- In the strong and weak isolation medium with incubation of 37°C for 18-24 h, the characteristics of Vibrio cholera colony is as follows:

- Colony in the Alkaline nutrition agar (AN): colorless, roundness, transparent or semitransparent, smooth surface, wet, flat or slight convex, neat edge and the diameter of colony is ca. 2 mm in general.
- Colony in the No. 4 agar (No. 4): the characteristics is similar with the colony in alkaline nutrition agar, but most of them show semitransparent; because these nutrient mediums contain tellurite, the center of colonies generally present grey or grey to black, and moreover the color become darker with the increase of cultivation time.
- Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.

- Slide agglutination test (ST): Pick suspected colonies from the isolation medium and pure cultivation of suspected colonies was conducted in the non-selective medium at 37°C for 18h~24h. Pure culture was slide agglutination tested with vibrio cholera O1 in serum and vibrio cholera O139 in serum, strain screening will be carried out. If the obvious visible aggregations appear in 1min, and there are no agglutinations in physiological saline, so the judgment is agglutination positive. On the contrary, the judgment is agglutination negative. Each sample should be selected at least more than five suspected colonies that be identified.

- Oxidase test (OT): Fresh cultures of growth on the isolation medium are daubed on a clean filter paper, and then add N,N-Dimethyl-p-phenylenediamine dihydrochloride or 1% N,N-Dimethyl-p-phenylenediamine dihydrochloride. If cultures become pink-purple-blue purple in 1~2 min, and some are purple at last. The judgment is oxidase test positive. If cultures didn't color, the judgment is oxidase test negative.

- The experimental results are filled in the following sheet.

NOTE: If finding positive strains in slide agglutination test and oxidase test, all the positive and suspicious strains should be transported to Shanghai Disease Control Centre immediately to do further detection.

6. Method References

Diagnostic criteria of Vibrio cholerae (WS289-2008).

Vibrio cholera determination log sheet

Sample No.	Time		The volume of water sample (mL)	Isolated culture (color reaction)		Positive or negative in Strain identification	
	Sampling time	Detection time		AN	No.4	OT	ST

Alkaline nutrition agar (AN): colorless, roundness, transparent or semitransparent, smoothsurface, wet, flat or slight convex, neat edge and the diameter of colony is 2 mm in general.
No. 4 agar (No. 4): the characteristics are similar with the colony in alkaline nutrition agar, but they have badly transparent and most of them show semitransparent; the center of colonies generally presents grey or grey to black, and moreover the color become darker with the increase of cultivation time.
ST— Slide agglutination test (agglutination positive or agglutination negative)
OT—Oxidase test(positive or negative)

Remarks:

Analyst: Assessor: Approver:

Appendix G Standard Operating Procedure for Particulate Organic Carbon (POC) Analysis

1. Method Overview

Spectrophotometry measures the decreased absorbance of dichromate solution to determine the amount of oxidizable carbon. The reaction is wet oxidation between acidic dichromate and carbon. The measurement range (measured as C) is 0.01 - 4 mg/L.

2. Method References

Spectrophotometry is adopted as the testing method in accordance with Gulf ecosystem observation methods (China Environmental Science Press, 2005/4.5.14.1)

3. Instruments and Reagents

3.1 Spectrophotometer

3.2 Filter membrane: 0.45 μm of pore size, GF/C glass fiber filter membrane (diameter is 47 mm) and standard micropore filter equipment. Filter membrane should be packed inside aluminum foil and placed into muffle furnace for 24 h heating at 450~500°C to remove oxidizable substances (the temperature should be not more than 500°C; otherwise the filtration ability would be changed).

3.3 Sulfuric acid/dichromate oxidant: dissolve 4.84 g potassium dichromate ($K_2Cr_2O_7$) in 20 mL distilled water, and put this solution slowly into 1000 mL volumetric flask which has 500 mL concentrated sulfuric acid (AR). After the mixed solution cools down, fix volume of 1000 mL volumetric flask by adding concentrated sulfuric acid, and then preserve this solution in glass bottle with stopper. This solution is stable.

3.4 Mixing cylinder with stopper: 50 mL

3.5 Volumetric flask: 1000 mL

3.6 Phosphoric acid (H_3PO_4, AR): 70%.

3.7 Sodium sulfate solution: dissolve 45 g sodium sulfate (Na_2SO_4) in 1000 mL distilled water.

4. Analytical Procedures

4.1 Put the pretreated glass fiber filter membrane in the standard micropore filter equipment. Connect filter equipment with vacuum pump that can control the vacuum degree. Pipette some seawater sample (0.5 - 2 L in general) to filter equipment. After filtration, the air suction of filter membrane lasts for 1 min. Add 2 mL sodium sulfate solution and then repeat air suction. Add 2 mL sodium sulfate solution again and take out filter membrane during air suction.

4.2 Put the filter membrane into a 30-mL beaker. Add 1.0 mL phosphoric acid and 1.0 mL distilled water into the beaker. Mix thoroughly. Put the beaker on heating plate for heating 30 min at 100-110°C. Beaker should be covered using glass dish when heating.

4.3 Add some sulfuric acid/dichromate oxidant and distilled water. The addition volume of oxidant can be determined by the expected carbon quantity as shown in the following table:

Evaluation of the dosage of oxidation

Expected carbon quantity (μg)	Oxidant (mL)	Distilled Water (mL)	Final Volume (mL)	Length of Cuvette (cm)
<300	2.00	0.8	100	10
300-700	4.00	1.6	50	2.5
700-2000	10.00	4.0	50	1

4.4 Mix thoroughly. Every beaker should be covered by glass dish and be heated at 100 - 110°C for 60 min.

4.5 Place cool, and move the solution and glass fiber filter membrane to the proper measuring cylinder. Distilled water is used to wash beaker wall and pour the wash water into the measuring cylinder. Plug the bottle tightly and mix upside down thoroughly. Waiting for the solution to cool down at room temperature and the filter membrane would sink to the bottom of the measuring cylinder.

4.6 At the wavelength of 440 nm, measure the decreased absorbance of blank solution using practical water sample as the reference solution

4.7 Because Cr III absorbs the decreased light, the measured absorbance should be calibrated by the following formula:

$$E = 1.1 \times E_f$$

where: E_f = the difference of decreased absorbance between practical water sample and blank solution in section 4.6.

4.8 POC (measured as C) could be computed by the following formula, μg/L:

$$POC = \frac{E * F * v}{V}$$

where: V = the volume of the filter sample, L; v = the required volume of oxidant in section 4.3; F = 275 (section 6.3).

5. Blank Test

Every unknown sample should carry out the blank determination using the filter membrane and oxidant. Using distilled water as reference solution, the decreased

absorbance of blank should be in the range of 1 - 1.1.

6. Calibration

6.1 Standard solution of glucose: dissolve 7.50 g glucose and some quantities of mercuric chloride crystal ($HgCl_2$) to form 100 mL solution. The stability of the solution could be preserved in freezer for several months. If the solution turns up turbidity, it cannot continue to be used.

6.2 Add distilled water to dilute the solution from 10 mL to 1 L so that 1.00 mL solution has 300 μg C.

6.3 Put one piece of glass fiber filter membrane and 1 mL phosphoric acid into a 30 mL beaker. Heat them at 100 - 110°C for 30 min. Add 10 mL oxidation and 4 mL dilute glucose solution into every beaker. Following the steps in sections 4.4 - 4.7, factor F could be computed as follows:

$$\mathrm{F} = \frac{120}{E_3}$$

where: E_3 = the average decreased absorbance of the calibrated Cr III at 440 nm wavelength. The computed result of F is about 274.

Particulate organic carbon (POC) analysis log sheet

Sample No.	Time		E_f	V	ν	F	POC (mg/L)
	Sampling time	Detection time					
Calculation formula:							
$POC = \frac{E * F * v}{V}$ E = 1.1* E_f F = 274 V = the volume of the filter sample (L) ν = the required volume of oxidant (mL)							

Analyst: Assessor: Approver:

Appendix H Standard Operating Procedure for Total Suspended Solids (TSS) Analysis

1. Method Overview

When the certain amount of water pass through 0.45 μm filter membrane, weigh the suspended matter which left on the filter membrane to calculate the concentration of suspended matter in water.

2. Method References

The specification for marine monitoring – Part 4: Seawater analysis (GB 17378.4-2007).

3. Analytical Procedures

3.1 Filter membranes are baked at 40-50°C and keep them at constant temperature for 6 - 8 h. After that, take them into the silica gel drier for 6 - 8 h.

3.2 Count the number of blank calibrated membranes and point color dot on membrane to distinguish the filter membranes of water samples.

3.3 Weigh filter membranes and put them into the numbered filter membrane boxes.

3.4 Use stainless steel tweezers to put the weighed water sample filter membrane (W_2) on the weighed blank calibrated membranes (W_b), and place both of them into filter.

3.5 Mix water sample thoroughly. Use measuring cylinder to take a certain volume of water sample (If the concentration of suspended matter is greater than 1000 mg/L, take 50 - 100 mL of water sample; if the concentration of suspended matter is less than 100 mg/L, take 1 - 5 L of water sample).

3.6 Turn on vacuum pump. Pour water sample into the filter. Use distilled water to wash the measuring cylinder and the washing water is also poured into the filter. In order to wash away salinity, after pumping water out at the first time, use distilled water to wash filter membrane for three times (50 mL per each time), and then pump water out again.

3.7 Use stainless steel tweezers to take the filter membranes back to their original filter membrane boxes. Either place the boxes into oven and dry them at 50°C, or dry them by air drying in natural environment. Cap the filter membrane box and preserve them in order. Bring these boxes to laboratory.

3.8 Drying: place filter membranes into the electro-thermal constant temperature dry box (40 - 50°C) to dewater filter membranes at constant temperature for 6 - 8 h. Thereafter, take filter membranes out and place them into the silica gel drier. After 6 - 8 h, weigh them (W_1).

3.9 Weighing: select the sense of analytical balance on basis of the quality of suspended

matter. If the quality is less than 50 mg, select the one hundred thousandth balance; if the quality is greater than 50 mg, select the one ten thousandth balance. Weighing should be quickly. Two times of weigh, before and after filtration, should keep the similar conditions in terms of temperature and humidity in balance room.

4. Blank calibration of filter membrane

4.1 During filtration, the glass fiber filter membrane might be dissolved leading to weight loss. Therefore, it must carefully deal with the weighing work of blank calibration sample.

5. Data Analysis and Calculations

5.1 The concentration of suspended matter should be calibrated by the following formula:

$$\rho = \frac{W_1 - W_2 - \Delta W}{V}$$

where: ρ = the concentration of suspended matter, (mg/L); W_1 = the total weight of suspended matter, water sample and filter membrane, (mg); W_2 = the weight of water sample and filter membrane, (mg); W = the calibration value of blank calibration sample, (mg); V = the volume of water sample, (L).

The calibration value of blank calibration sample can be calculated by the following formula:

$$\Delta W = \frac{1}{n} \times \sum^{n} (W_n - W_b)$$

where: W_n= the weight of the blank calibration filter membrane after filtration, (mg); W_b= the weight of the blank calibration filter membrane before filtration, (mg); n = the number of the blank calibration filter membrane; ΔW should be negative number.

Total suspended solids (TSS) analysis log sheet

Sample No.	Time		Filter weight (mg)		V (L)	TSS (mg/L)
	Sampling time	Detection time	Before filtration (W_1)	After filtration (W_2)		
Calculation formula:						
$\rho = \frac{W_2 - W_1}{V}$ ρ = Total suspended solids concentration, (mg/L); W_1 = Membrane weight, (mg); W_2 = Membrane weight, after filtered, (mg); V = The volume of filtered sample, (L).						

Analyst: Assessor: Approver:

Appendix I Standard Operating Procedure for Salinity Analysis

The testing method use SYA2-2 salimeter to determine salinity of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/29.1).

1. Scope and Application

This method is applicable to salinity determination of seawater on land or in the laboratory on board.

2. Method Overview

Use inductive salinity meter for determination.

3. Reagent

Standard seawater.

4. Instruments and Equipment

Main technical indicators of inductive salinity meter: conductivity ratio 0.07-1.2; measurement accuracy is 0.01; measurement precision is 0.003; salinity resolution is 0.001; accuracy of temperature measuring of electrical bridge is 0.5°C.

5. Procedure

5.1 Preparation: Place the water sample until the temperature difference between the sample and standard seawater is within ±2°C to prepare for measurement.

5.2 Temperature and salinity measurement inspection

5.2.1 Turn the temperature-salinity switch to “temperature measurement”. Compare the temperature reading with the room temperature. If the deviation is lower than ±1°C, the temperature bridge operates normally.

5.2.2 Tighten the drainage knob below the water cup. Place the seawater whose salinity is known to the inlet below the conductivity cell and turn the clock of conductivity cell to “inlet”. Open the air pump switch. Press the gas hole on the water cap tightly with the middle finger of the left hand, and at that time, the seawater will flow into the conductivity cell slowly. When a little seawater overflow from the outlet of conductivity cell to the water cup, turn off the cock of conductivity cell immediately, release the finger and close the air pump. The conductivity cell is filled with the seawater at that time.

5.3 Calibration

5.3.1 Fill the conductivity cell with the standard seawater slowly. Wash the cell for 1-2 times, and then measure the temperature of standard seawater and write down the data on datasheet.

5.3.2 Look up the corresponding R2 value according to the temperature conversion table on the instrument panel. Rotate R2 knob to this value and record R2 value.

5.3.3 Shift the temperature-salinity switch to “salinity measurement” and adjust R1 knob to make zero-header point at “zero”. Stop stirring and drain water. Repeat filling adjustment until the reading repeats, then the calibration is completed. Record the R1 value.

5.4 Water sample measurement

Start up the air pump. Pipette the water sample into the conductivity cell and wash for 1-2 times. When the water sample overflows from the conductivity cell, turn off the inlet cock of conductivity cell immediately, cut down the power of the air pump and start mixing. Turn temperature-salinity switch to “temperature measurement”. Measure the temperature of the seawater sample and write down the data in the datasheet. Shift temperature-salinity switch to “salinity measurement” and adjust Rt knob to make zero-header point at “zero”. Stop stirring and drain the water sample in the conductivity cell. If the change of last digits of conductivity ratio knob in two measurements is less than 6, these two measurements are regarded as repeat. Record the conductivity ratio R1 value of the seawater sample.

6. Computing and Reporting

According to the formula, programming should be relied on for calculation. The result should display three digits after the decimal point.

7. Attentions

- 250 mL sampling bottle and the plug should be washed for 3 times with the same water sample before use. The used sampling bottle should be filled with a little seawater but drained the water at next sampling time;
- When fill the conductivity cell with the seawater sample, avoiding the bubbles in the conductivity cell. If there are bubbles, the reading will be lower in general. At that time, the sample should be re-filled and measured again;
- When fill the conductivity cell with the seawater sample, drain the residual water sample inside inflow tube and dry the tube. After that, measure the sample according to the steps described. Otherwise, the residual water will pollute the water sample.

Salinity analysis log sheet

Date: Operator: Approved by:

Project name		Project ID	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH	Detection date and time	
Instrument	Salimeter		
Instrument model	SYA2-2		
Standard value of standard seawater:	R_{15} value of standard seawater:	Control standard seawater (detection):	

Test Results

Sample ID	Salinity (PSU)	Sample ID	Salinity (PSU)

Remarks:

Appendix J Standard Operating Procedure for Temperature Analysis

The testing method use Surface Water Thermometer to determine temperature of water sample in accordance with the Specification for Marine Monitoring - Part 4: Seawater Analysis (GB17378.4-2007/25.2).

1. Analysis method

When using the surface water thermometer, tie the haul loop on top of the metal pipe with rope firstly. Use bucket to collect water sample. Put the surface water thermometer into the bucket during measurement. After 1-2 minutes, empty the water in the bucket and thermometer tube. Collect the water sample and put the thermometer into the bucket again. Read the temperature after responding for 3 minutes and read the temperature again after another 1 minute. When the air temperature is higher than the water temperature, calibrate the smaller one of the readings to get the measure value of surface water temperature. On the contrary, calibrate the bigger one of the readings to get the measure value of surface water temperature.

2. Attentions

- When reading the data, the sight line and the top of the capillary of the surface water thermometer should be at the same level. Direct sunlight should be avoided.
- The bucket is better to be made of heat-insulation materials. The capacity is about 5-10 L.
- Surface water thermometer must have periodic verifications according to the specification.

Temperature analysis log sheet

Date: Operator: Approved by:

Project name		Project ID	
Date of consignation		Sampling date	
Standard	The National Standard of the Specification for Marine Monitoring-Part 4: Seawater analysis		
Testing condition	Temperature: °C Humidity: %RH	Detection date and time	
Instrument	Thermometer		
Instrument model			

Test Results

Sample ID	Temperature (°C)	Sample ID	Temperature (°C)

Remarks

Corrected value of thermometer			
Indicating value (°C)	0	20	40
Corrected value (°C)	0.36	0.46	0.62